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AN ANATOMICAL STUDY OF FIFTEEN CASES OF ACUTE POLIOMYELITIS.*

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From Heine's deductive guess at the nature of the pathology of Poliomyelitis in 1860, to the description of the lesions in a case autopsied seven hours post mortem by Drummond in 1885, investigators had had only chronic changes to report. These early workers included Cornil,[1] Charcot and Joffroy,[2] Roger and Damaschino,[3] Gombault,[4] Dejerine,[5] and others.

Drummond[6] described the macroscopic congestion and minute hemorrhages in the cord. By him also were pointed out the degenerative changes in the ganglion cells and the leucocytic infiltration. Rissler[7] in 1888 was the first to show the involvement of the pia mater. Batten[8] in 1902 concluded that the anterior horn change was thrombotic in origin. Among others who have supported this view are Money, Marie, and Hock.

In 1905 Wickman[9] published his findings in seven recent and two old cases. His was the first extensive series. He called attention to the presence of normal ganglion cells in the neighborhood of altered vessels. He also suggested the lymphogenous nature of the infection.

Barnes and Miller[10] in 1907 reported one case and described the nature of the infiltrating cells. In the same year Harbitz and Scheel[11] also published a very complete

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study of fifteen cases. They thought the process began in the pia mater and extended to the cord, with the usual perivascular, infiltrating, and ganglion cell changes. They also described the wandering cells in detail, and believed that the polymorphonuclear leucocytes were to be found chiefly in the anterior horns as opposed to the pia mater. They found no lesions in the peripheral nerves, and held that with the exception of bronchopneumonia the organs outside of the central nervous system were slightly affected.

In 1909 Zappert[12] reported his study of the Austrian epidemic of 1908 describing a "hyperplasia of the entire lymphoid apparatus." The year following, Ed. Müller's[13] findings in seven cases of the Hesse-Nassau epidemic of 1909 were published. He regularly found bronchitis and enlargement of the spleen. One of his cases had enlarged tonsils.

Robertson[14] reviewed the literature in 1910 and added six new cases. He emphasized the presence of hemorrhage into the cord, advancing it as a cause of fatal result. He thought there was a lymphatic hyperplasia throughout the body. The same year Strauss[15] reported eight cases, and gave an excellent description of the disease and its pathology. He considered the vascular mantling periadventitial rather than located in the perivascular lymph spaces and that the glia cells were the origin of the wandering cells.

Peabody, Draper and Dochez,[16] in 1912, reported autopsy findings in eleven acute cases. They laid particular stress on the visceral changes which they felt had been slighted by previous writers. They noted that the liver was constantly the site of focal necroses, and that cloudy swelling occurred in all the parenchymatous organs. They often found the glands and tonsils enlarged, and Peyer's patches in the intestine were usually swollen. The spleen was enlarged and showed prominent follicles.

Finally, in 1913, Wickman[17] published an exhaustive monograph, in which the pathology was clearly set forth. In this paper attention was again directed to the disease as a general infection. Enlargement of the spleen and hyperplasia of the intestinal lymphoid tissue was found. He

showed that polymorphonuclears were rarely found in the pia mater, and that changes of a vascular nature chiefly involved the veins. He found no changes in the dura mater.

The following description is based on the material obtained from fifteen autopsies on acute cases of Poliomyelitis studied in the laboratory of the Presbyterian Hospital during the fall of 1916. Thirteen of these cases occurred in the recent epidemic. Two had died previously. Eight of the cases were from the Willard Parker Hospital. Through the courtesy of Drs. Anna Williams and H. L. Abramson of the New York Health Department, we had access to this material. Three cases were from the Presbyterian Hospital and one from the Roosevelt Hospital. The remaining were outside cases. In two of them our material was limited to the spinal cord, but we were able to make brief reports of the gross pathological findings in the other organs.

All of the fifteen cases were children from seven months to ten years of age. The majority were under three years. At autopsy the bodies were usually found well developed and well nourished.

As routine, paraffin mounted hematoxylin and eosin stained sections were used. For study of the nervous system we employed successfully Noguchi's and Lenhossek's stains, also in special instances Van Gieson's, Giemsa's, Gram, Marchi, and the Sudan III stain. Protocols of the cases are omitted for lack of space.

Brain and Cord. — The gross appearance of the brain was not characteristic. But in a typical case the cord lesions were rather striking. In the literature there is some uncertainty as to whether or not the spinal fluid is increased. In all of our cases the normal quantity was found, under no apparent excess of pressure. The pia covering both the brain and cord often appeared edematous. The brain and cord were frequently quite soft, and there was almost regularly a surface injection of the vessels. On section nothing was to be made out in the brain beyond congestion, which

sometimes was marked. Section of the cord on the contrary often showed the horns as prominent dark gray or pink H-shaped areas, frequently speckled with bright red pin-point dots. In only one case were we able to make out a distinct softening of the gray matter such as Harbitz and Scheel have described. This occurred in the lumbar segment on one side, and after formalin fixation was the site of a definite cavity. However, certain cut sections which grossly exhibited no change have proved to be the scene of extensive microscopic lesions. The whole cord on section had a tendency, as though it was under tension, to project beyond the cut margin of the pia mater, indicating a swelling which was probably due to edema and congestion.

Membranes: No changes have been seen in the dura mater. The pia mater covering the brain has been normal except in three cases (Nos. 8, 13, 15). In one we were able to see grossly a thickening and opacity of the membranes, chiefly where they dipped into the fissures. Microscopically this case showed a meningitis over the whole brain, including the cerebellum. The predominating cell in the exudate was the polymorphonuclear leucocyte, but there were also many mononuclears. With a Giemsa stain we were able to demonstrate a probable organism in the exudate which we shall refer to under a different heading. In the second case the meninges were markedly raised from the surface of the brain, as though by edema. A fair number of large mononuclear cells and red blood corpuscles were found under the meninges.

The pia mater of the cord has regularly been the seat of diffuse and perivascular-cell accumulation. It was most infiltrated where it extended into the ventral fissures of the cord, and in general also over its anterior surface. The nature of some of these cells was not always certain. The prevailing cell as a rule was a mononuclear. Some were not to be distinguished from lymphocytes, some showed larger and less dense nuclei, which were oval or round in form. Still other cells occurred with a faint cytoplasm and large

oval vesicular nucleus resembling endothelial cells. Occasional plasma cells were to be observed. In some cases polymorphonuclear leucocytes were numerous. In other cases they were rare. The oxydase reaction was useful in determining the exact amount of granular cell invasion. Finally, the vessels of the pia mater were as a rule extremely engorged.

Cord. — Vascular changes: These may be the chief feature of the microscopic picture, or they may share about equally with the ganglion cell change and diffuse infiltration of the horns; rarely were they relatively inconspicuous. The vessels were seen to stand out as bright red channels crowded with red blood corpuscles. About them was a dense mantling of cells. These cells for the most part had single small darkly staining nuclei, often distorted in shape from the pressure of being closely packed together. Among them here and there, in some instances, one could see large cells with round, oval, or irregular nuclei showing little chromatin. These cells all appeared to be of the same origin. We were not impressed by the presence of many polymorphonuclear leucocytes in this location until we applied the oxydase reaction. In the majority of instances this reaction disclosed a considerable number of the granular cells which had formerly escaped notice.

The injected vessels rarely showed any disproportionate increase of white blood corpuscles and seldom a migrating cell in their walls; while thus giving little support to the idea that these cells had their origin through the blood this observation is not conclusive. With Van Gieson's stain we were able to make out that the greater portion of the mantling was outside of the adventitial coat of the vessel wall. This was pointed out by Strauss and is contrary to the generally accepted view that the perivascular lymph spaces lying between the muscular and adventitial coats of the vessel are the sole scene of the round-cell accumulation. Mantling of the vessels occurred quite as extensively in the posterior as in the anterior horns. In the white matter the vessels were

as regularly affected, but the greater vascularity of the horns appeared to cause the more striking effect in them.

Ganglion cells: The ganglion cells showed the greatest variety of change from the slightest recognizable injury to entire disappearance. The earliest changes as demonstrated by Strauss were the destruction of the fine neurofibrils of the nerve cell proper. The sequence of events, as we were able to make it out, following this was altogether uncertain. The nerve cells swell, lose their Nissl bodies, and stain with difficulty. Their cytoplasm sometimes appears granular and neuronophagia seems to be a relatively late feature of the process. All stages of neuronophagia were to be made out from a barely recognizable increase of neighboring wandering cells, to a disintegrating cell lacunated and covered by phagocytes. In what must have been still later stages one saw nothing but a mass of the scavenger cells accumulated at points which evidently had been the site of ganglion cells.

Often the early changes were manifest in a peripheral collection of the Nissl bodies. This seemed particularly true of the cells of Clarke's column, which were relatively immune and rarely showed extreme injury. We agree with Strauss that vacuolization is extremely rare, but nuclei eccentrically placed were more frequently observed than he has admitted. The nucleus was generally the last element of the cell to show signs of injury. It often appeared unchanged in a cell whose cytoplasm stained with the greatest difficulty, or showed no Nissl bodies. Often the nuclei remained with just a rim of ragged cytoplasm about them. In other instances nothing but the nuclei were left. This was not always true because in some instances one found the pale swollen ganglion cells showing only a faint nucleus or none at all. Obviously degenerated ganglion cells often exhibited no signs of neuronophagia. When the process was present the type of cell was usually the mononuclear, though in certain instances the polymorphonuclears seemed to outnumber them. The mononuclears observed about such a cell were of many varieties, appearing not unlike the cells to

be described under the diffuse infiltration. Their nuclei ran the gamut of shape and size from the small pyknotic types to others with irregular shapes and a fine chromatin network. Occasionally one saw in such an area a horseshoe-shaped nucleus pushed well to the side of the cell with a cytoplasm evidently stuffed with recently ingested material.

As with the other changes in the cord different levels and different sides at the same level exhibited impressive disparities in the intensity of the ganglion-cell injury. We were struck by the number of times relatively uninjured ganglion cells were to be found in the ventral groups of the motor horns. The greatest and most constant injury was seen in cells located in the center of the horn and in the neighborhood of the commissure.

Römer[18] has pointed out that the ganglion cells are the seat of three types of injury. He describes them as either entirely disappeared, as washed out and homogeneous looking, or finally as showing neuronophagia.

Very remarkable were ganglion cells in which no change by Lenhossek's stain were to be made out, found occurring side by side with other ganglion cells exhibiting extreme injury and lying in a region of advanced cellular infiltration. On the other hand, we have been able to see disintegrating cells in sections which were strikingly free from infiltration. This is contrary to the view held by Wickman that ganglion cell changes are not observed where there is no interstitial change. From these observations we are inclined to the older view, held by Charcot and Joffroy, Gombault, Dejerine, Rissler, and more recently by Bruining,[19] that the ganglion-cell injury is not necessarily secondary to the interstitial changes of edema, congestion, and cell accumulation as elsewhere described, but is probably the result of direct injury by the causative organisms or their toxins. This is contrary to the most recently expressed opinions of Goldscheider,[20] Starr,[21] Taylor,[22] Wickman, Strauss, and others.

Interstitial changes: A diffuse and focal infiltration was another characteristic lesion of both the anterior and posterior horns. The focal cell collections we have described

elsewhere as accumulated at the site of the deceased ganglion cell. The posterior horns have usually been affected along with the anterior, but the process has as a rule confined itself to the ventral half of the horn. The type of cell making up this infiltration was again the mononuclear. The most striking of these cells possessed a large curiously crooked and elongated nucleus without a dense chromatin content. Some of the cells were quite large and often the section was largely overrun with them. Small mononuclears of the lymphocyte type were generally abundant. Ranging between these and the cells with crooked elongated nuclei were many wandering cells presenting nuclei of various forms, and strongly suggesting intermediate stages in the transition of one cell to the other. Wickman advances this idea, holding to the opinion that the large cells are polyblasts of Maximow,[23] and represent a further stage in the development of the lymphocyte. Large vacuolated cells occurred having a horseshoe-shaped nucleus near the cell border. These cells were fairly abundant in some sections, while absent from others. Fairly numerous also were cells with large oval or round vesicular nuclei displaying a very delicate chromatin network.

In some cases polymorphonuclear leucocytes appeared to be quite numerous, but in view of the number of cells with irregular and uncertain nuclei it was often difficult to decide whether we were dealing with cells of the granulated series or not. To decide this point we employed the oxydase reaction as described by Winkler[24] and by Evans.[25] It was fairly simple by this method to see at a glance how prominent a part the granular cells played in the reaction. In some of the cases they were quite, if not very numerous. In others it was rare to find a cell giving this reaction. In one case of thirteen days' duration no polymorphonuclear leucocytes were found, tending to confirm Robertson's contention that these cells appear early and then disappear. However, another case of only thirty-six hours' duration showed the absence of polymorphonuclears in the cervical and thoracic sections, though a fair number were present in the lumbar

segments, in spite of marked lesions at all three levels. The process in the lumbar cord in this case may have represented the most recent lesion. Other cases that showed many polymorphonuclears were of two or three days' duration, while still others with just as brief a period of illness showed relatively few or no polymorphonuclear leucocytes. Harbitz and Scheel have described these mononuclear cells in some detail placing them in four categories, according to the form and position of the nucleus. Opinion has been considerably divided over the origin of the diffusely infiltrating cells. By Rissler, Harbitz and Scheel, and others they have been held to be leucocytes. Goldscheider and Strauss consider them of glial origin. Robertson believes that the mitotic figures he has seen in the endothelial cells indicate these as the cells of origin. Marburg [26] holds that they are lymphocytes. Wickman is inclined to regard all of these cells as of the polyblastic series.

We share with Wickman the idea that the lymphocyte is the first arrival and from it the other cells are derived. This is supported by Maximow's experiments showing that lymphocytes can assume polyblastic characteristics in certain types of reaction. Further evidence is afforded in the remarkable variety of transition forms to be made out in each section.

Hemorrhage: Hemorrhage has occurred rather infrequently in this series of cases and has never been conspicuous. Wickman, Harbitz and Scheel, Strauss, Robertson, and others have described it more regularly, the last author laying great stress on it as a factor of cause in the symptomatology and fatal results.

White matter: In cases of well-marked vascular change, the white matter strikingly shared in the process. The vessels were congested and surrounded by collars of round-cell accumulation. It never seemed possible that these mantling cells here or in the gray matter were capable of producing

pressure effects sufficient to mechanically obstruct the vessels and thus bring about an anemia which would be sufficient cause for the ganglion cell degenerations. In fact, such mantled vessels usually presented wide lumina engorged with blood, which was hardly compatible with the idea of compression as advanced by some experimenters, particularly Peabody, Draper and Dochez. In two or three instances focal infiltration was seen, but it occurred in that part of the white matter in immediate juxtaposition to the anterior horns. Diffuse changes did not occur. The very loose nature of the tissues in certain cases pointed to edema of the cord as a distinct feature of the lesion. Wickman thinks that the absorption of this accounts for the rapid subsidence of the symptoms. Using the Marchi method, neither Wickman nor Strauss was successful in demonstrating degeneration in the fiber tracts of the cord. In nine cases in which we also attempted to show degeneration by that method we obtained negative results in all except one. Here there was slight degeneration only.

What segment of the cord is most severely injured is a question that has long been asked. In this series the cervical cord was generally more seriously involved than the lumbar segments. In the thoracic region the lesion was sometimes quite severe, but rarely as marked as in either of the enlargements. In every case the cervical cord showed lesions. In four cases it was difficult to make out any change in the lumbar segments, while the cervical cord was the seat of a rather widespread injury (Nos. 3, 4, 8, 11). The thoracic cord was very slightly involved in six (Nos. 2, 5, 7, 10, 12, 14).

Basal ganglia and pons: In these locations there were usually changes to be made out. These were most often a ganglion-cell degeneration with disappearance of the Nissl bodies, pallor of the cytoplasm, and an eccentric nucleus. Perivascular infiltration was less regularly present in these higher levels. It was quite rare to find a diffuse scattering of wandering cells. In none of the twelve cases in which the

cerebellum and cerebral hemispheres were studied could one make out any changes save those of congestion. The hypophysis in two instances was normal.

Posterior root ganglia: Harbitz and Scheel found these negative. Peabody, Draper and Dochez, Strauss, and others usually saw the characteristic changes described in the cord.

Peripheral nerves. — Redlich [27] and Mönckeburg [28] were able to show slight degenerative changes by the Marchi method. Strauss in one case was able to demonstrate degeneration in the roots. The literature is barren of other efforts along this line. In one of four cases in which we were able to study the peripheral nerves many of the fibers were degenerated, and this was evidenced in the fragmentation of the myelin sheath which stained black with osmic acid. This patient died thirty-six hours after onset and it was apparently too early for degenerative changes to manifest themselves as the result of the destruction of the cells. In view of the scattered nature of the lesion in the anterior horn at different levels in most cases it does not seem strange that a chance examination of one or two nerves should disclose nothing. More thorough study of the peripheral nerves in all cases would undoubtedly give more positive findings.

The heart and vessels appeared normal in every case.

The lungs in six cases out of twelve showed a moderate degree of bronchopneumonia. Congestion was a feature in only three. There was an acute fibrinous pleurisy in one case, and in only one was edema marked. Other than this the lungs showed no pathological change.

The thymus to gross examination always appeared normal. In only one instance was it noted as enlarged. Microscopic study showed evident regressive changes in the majority of cases, which was not in keeping with the hyperplasia observed

by Peabody, Draper and Dochez. This change was particularly marked in two cases. The small thymic cells often showed signs of injury and the large phagocytic mononuclears were sometimes vacuolated and in other instances contained nuclear débris in great quantities. Pappenheimer[29] in his careful study of the thymus makes it clear that this organ is peculiarly susceptible to injury, and regressive changes set in with surprising rapidity in both acute and chronic infections.

The lymph nodes were usually quite red both on the surface and on section. There were never any palpable nodes in the axilla or groin. The mesenteric nodes in six instances were noted as slightly enlarged. Twice they were definitely larger than normal. In the remaining cases they were unchanged in size. At autopsy these alterations never seemed important when the normally large size of the mesenteric nodes of the child was recalled. Microscopically the picture was similar to that to be described in the splenic follicles, but less marked. Edema and congestion seemed sufficient in most cases to account for the slight enlargement of the glands.

The tonsils from four cases were studied. Twice they were apparently normal to both microscopic and gross study (Nos. 12 and 13). In the other two (Nos. 3 and 4) there was a moderate amount of necrotic material in the crypts with a corresponding degeneration of the epithelium. The centers of the follicles showed an apparent increase in the large mononuclear cells exhibited generally by the lymphoid apparatus. Harbitz and Scheel found the tonsils normal, along with the nose and throat in all of three cases examined. Peabody, Draper and Dochez thought that the tonsils were usually larger than normal. Wickman and others fail to touch on this point specifically.

The spleen was entirely unlike what we are accustomed to see in this organ in most acute infections, in that it was

never soft and usually noted as rather firm. As a rule it was not enlarged. On section the follicles had a tendency to stand out prominently above the cut surface as white translucent dots. There were several cases, however, in which the follicles were most inconspicuous. In some the organ seemed to be extremely congested.

Microscopic examination has been focused on the follicular changes and most observers have called attention to a hyperplasia. Peabody, Draper and Dochez described endothelial proliferation at the follicular center and in the sinuses. We have been able to make out three degrees of change in the follicles. In seven instances the follicles were strikingly small and hypoplastic. In others the large phagocytic mononuclears were very prominent. These cells contained large oval or round vesicular nuclei with a loose chromatin structure and showed a faintly staining though abundant cytoplasm. In still other cases there were definite hyaline changes at the center. The large nuclei of the cells described, with a variable amount of nuclear detritus, appeared imbedded in what was obviously the fused cytoplasm of the disintegrating large mononuclears. Frequent evidence was to be seen of the destruction of lymphatic elements in their pyknotic nuclei, fragmentation, and ingestion by phagocyting cells. In general the spleen showed a lymphatic hypoplasia rather than a hyperplasia.

Liver: Peabody, Draper and Dochez in their monograph, described at some length most striking changes in the liver, and Batten[30] has accepted their view without mentioning whether or not he was able to see the same thing. The lesions are described as focal necroses, characterized by liver cell degeneration with lymphoid and polymorphonuclear infiltration. Such changes were noted chiefly about the central veins, and they are described as sometimes difficult to find, in other cases many of them occurred in a single low power field.

Other observers have been content to point out merely the changes characteristic of cloudy swelling in a parenchymatous organ. In our series no instances of focal necrosis

were found. In one case only did we see even a moderate number of polymorphonuclear leucocytes about the portal spaces. But many cases of acute infection and other diseases present this picture about the vessels of the liver. Stained by Sudan III the liver cells were regularly found to contain quantities of fat deposited in fine vacuoles. In several cases the liver shared with spleen and lungs in a distinct central vein stasis. Finally, as a rule, there was a moderate parenchymatous degeneration evidencing itself in swelling and the cloudy appearance of the liver epithelium, but this was not a prominent feature as one sees it in pneumonia, sepsis, and other acute infections.

The stomach was normal in all fifteen cases. There were no lesions in the intestines except in the lower end of the ileum. Occasionally there was no change at all, but the Peyer's patches were sometimes markedly enlarged, raised, and red. The mucous membrane was always intact over these areas. Microscopically in the submucosa engorgement of the vessels was the rule. The follicular changes were not unlike those observed in the spleen and lymph glands. In a few instances large mononuclear phagocytes were very numerous and active. But here again it was difficult to believe that there was a hyperplasia of the lymphoid structures. The very increase of the phagocytic cells was considered indicative of injured and disintegrating lymphocytes, for the removal of which they were obviously mobilized.

While superficially bearing some resemblance to typhoid lesions, the injury was never so severe. Ulceration did not occur and the enlargement of Peyer's patches was rarely impressive. When compared to the central nervous system, where the organism evidently does occur, the intestinal as well as other lymphoid lesions seem insignificant and hardly bespeak the presence of many, if any, organisms growing in these localities.

The kidneys always showed parenchymatous swelling and early degenerative changes. No cases of acute nephritis were found.

The adrenals, thyroid, and pancreas were apparently normal in all cases examined.

Organisms. — In the meningeal exudate covering the cerebrum and cerebellum of Case 8 we were able to demonstrate a few bodies suggestive of organisms. They stained by Giemsa and were of varying size. They were cocci as a rule. Some were quite small and appeared in chains. Others were seen singly or in pairs. They were not numerous. This was the only case to show a marked meningitis over the brain with a large number of polymorphonuclear in the exudate. The possibility of a secondary infection could not be excluded. Flexner and Noguchi[31] searched sections of the cord for organisms, and report finding them with difficulty in a few instances. In smears of the fresh cord they found them with greater ease. Using their stain we also studied numerous sections of the cord in an attempt to find and identify organisms. It was extremely difficult to feel convinced of having found any organisms in any of the cords we studied. They were never numerous, and the question of whether or not we were dealing with nuclear fragments, cross sections of nerve fibers, or small precipitations of stain, was never satisfactorily disposed of. There were, however, made out in several cases small bodies which varied considerably in size, and generally occurred in pairs, though also singly. These were seen in the anterior horns in the regions of greatest infiltration. Harbitz and Scheel, Wickman, and Robertson, were unable to identify any organisms in the cords of their cases.

SUMMARY.

Brain and Cord. — The ganglion cells were the scene of the most important changes. Injury to these cells appeared to occur early and was often so severe as to cause their total destruction. There appeared to be evidence, in comparing ganglion cell changes with interstitial alterations, of the truth of the older view that these cells suffer with the first injury

of the central nervous system. Among the interstitial changes in the cord the presence of many cells with single elongated, often quite crooked nuclei, produced a pathological picture peculiar to this disease. The cells entering into the perivascular accumulations contrasted sharply with the cells of the more diffuse infiltration, having as a rule small dense nuclei. The mechanical factors edema, congestion, compression, and the invasion of the tissues by wandering cells did not appear to be primary. Hemorrhage in these cases did not seem to be the serious factor advanced by some investigators. Secondary degeneration of the myelin sheaths of the nerves probably occurs more frequently than has been supposed.

The cerebral cortex uniformly showed no lesions. The burden of the injury appeared to fall on the cord and the brain stem.

The injury to other organs while constant was not characteristic of poliomyelitis.

Lymphoid Apparatus and Thymus. — The lymphoid apparatus appeared to show an actual diminution of lymphocytes. The gross swelling and redness of the lymph glands and Peyer's patches was probably produced by edema and congestion rather than the active multiplication of cellular elements. The tonsils in two out of four cases examined were normal. The spleen was more characteristic to gross than microscopic examination. Strikingly constant was a firm organ with prominent though not enlarged Malpighian follicles. The thymus regularly exhibited regressive changes. There was no evidence of the hyperplasia that has been described in this organ.

Liver and Other Organs. — The liver displayed no special pathological change. In no cases were we able to discover the focal necroses that have been written about. Fatty infiltration, and a moderate degree of cloudy swelling were regular findings. The lungs in the majority of cases were the seat of scattered patches of evidently terminal pneumonia of

the lobular type. The remaining organs showed no characteristic pathological alteration.

Organisms. — A search for organisms in sections of the cord, while giving doubtful results, was suggestive at least in several instances.

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A CASE OF METASTATIC LEIOMYOSARCOMA PRIMARY IN THE UTERUS.*

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The tumor reported in the following pages is known under a variety of names, such as leiomyosarcoma, malignant leiomyoma, and myoma sarcomatoides. It belongs in the group of new growths included by the term leiomyoblastoma, which is defined by Mallory as " a tumor of mesenchymal origin of which the cells tend to differentiate into smooth muscle cells; " that is, the smooth muscle cell is the type cell of this group of tumors and distinguishes them from all others. The features which characterize a smooth muscle cell are the following: a spindle-shaped cell, containing a more or less rod-shaped nucleus and having at the periphery of its rather dense acidophilic cytoplasm a number of delicate myoglia fibrils, which fuse together at the ends of the cell to form coarser fibrils.

The leiomyoblastoma may grow at various rates of speed. The slow-growing form is commonly known as leiomyoma, the rapidly-growing form as leiomyosarcoma. In leiomyoma the differentiation of the smooth muscle cells is as perfect as in the normal muscle, but in the leiomyosarcoma the differentiation is often less complete. The cells are frequently larger than normal and rarely may become more or less spherical in shape. Rapid proliferation is shown by the presence of mitotic figures which may be very numerous.

The leiomyosarcoma arises most commonly in the uterus. Although histologically malignant it usually remains within the uterine wall, or less frequently spreads locally, probably through the lymphatics, as in a case recently reported by Lahm, where metastasis occurred in the broad ligaments, ovaries, and adjacent structures. Very infrequently cases

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of extensive metastasis to remote organs by way of the blood stream have been reported.

Before considering the present case a few facts gathered from the literature on leiomyosarcoma in general may be helpful. Its frequency is variously stated, but as routine histological diagnosis is becoming more common, the number of cases is certain to increase. Murphy, in a series of 1,000 cases of leiomyoma, found one and one-third per cent of the histologically malignant variety; Graves believes that four to five per cent is more nearly true. The laboratory records of the Boston City Hospital show a total of 827 leiomyomata examined in twenty years; of this number twenty-four (or 2.9 per cent) are histologically malignant. Zacherl, after a study of the literature, concludes that for every forty or fifty cases of carcinoma of the uterus there is one case of sarcoma.

The tumor usually appears after the menopause. Veit has collected a series of 438 cases in women varying from five to seventy years of age, in whom the greatest frequency was during the period from forty-five to sixty years.

Malignant nodules, although often associated with benign leiomyomata, are usually single and located in the fundus. The symptoms, beyond rapidity of growth, are not characteristic and an absolute diagnosis is possible only on microscopic examination. The prognosis is good if the tumor is removed early. In neglected cases, according to Meyer, death results from cachexia or sepsis.

Examination of the rather extensive literature on the subject of metastatic leiomyosarcoma has been unsatisfactory, owing to the lack of a definite standard for smooth muscle cells among pathologists. Many tumors, obviously of mesenchymal origin, which were primary in the fundus of the uterus and which metastasized by way of the blood stream to the lungs and liver, have been reported. It seems probable that the largest part of these tumors were true leiomyomata, because of the frequency of such neoplasms at the site of the primary growth. Discussion of

these cases without the actual tissue for study and comparison is of little value. At any rate, it is certain that a leiomyosarcoma giving rise to metastasis is rare, for this is the first case in the records of this laboratory during twenty years. Fortunately all the steps for the proof of the exact nature of the tumor were possible, and fulfilled in this instance.

Report of Case:

Patient, A. F., age 55, single, book-keeper, gives the following history: In 1911, about four years previous to her death, she complained of increased frequency of micturition and a sensation of fulness in the abdomen. She was seen by a number of physicians and the diagnosis of "uterine fibroids" was made. In October, 1913, a supravaginal hysterectomy was performed by Dr. C. H. Hare, at the Massachusetts Woman's Hospital. The uterus was found to contain a large subserous myoma which extended laterally into the left broad ligament. A separate mass smaller in size was also present in the left broad ligament. The tubes, ovaries, and appendix (which was also removed) were not remarkable. The patient made an uneventful recovery. The pathological description of this material has recently been furnished the writer. This report is as follows:

Oct. 23, 1913.—"Received. Uterus, supravaginal, with attached uterine myoma, measuring 25 x 18 x 18 centimeters in left broad ligament; and a separate mass 11.5 x 8.5 x 8 centimeters, which shows calcification of superficial layers in plaques. Tubes, ovaries, and appendix not remarkable."

"Diagnosis: Multiple uterine myomata."

In May, 1914, the patient had a small subcutaneous "fibroid" tumor removed from over the right sacroiliac synchondrosis. The author has not examined tissue from this nodule.

In June, 1914, the patient began to have terrific pain in her back between the shoulder blades, which forced her to give up her work. In December, 1914, she was admitted to the Massachusetts General Hospital. Physical examination showed the following findings:

Patient, a well-developed, well-nourished woman.

Lungs, clear.

Back, considerable kyphosis and lateral scoliosis of the 4th to the 10th dorsal vertebræ, with a sense of fulness and resistance to the right of the spine. Back covered with numerous "neurofibromata."

Extremities, knee-jerks, Babinski and Oppenheim reflexes, hyperactive. Ankle clonus more marked on the right. Rough sensation. Pinpoint and head show acute sensation to cease at the level of the 9th or 10th dorsal segment. Anesthesia corresponds to the 4th dorsal segment. Several small subcutaneous nodules were removed from the back and a diagnosis of "malignant leiomyoma (sarcoma)" was made by Dr. W. F. Whitney.

On December 10, 1914, Dr. W. J. Mixter exposed the spinal cord from the 7th cervical to the 3d dorsal vertebra, removing laminæ and spinous processes. Cord normal to palpation. At the level of the 1st dorsal vertebra was an eliptical tumor mass 2.5 x .5 centimeters in diameter, lying to the left of the cord and displacing it slightly to the right. As the cord was retracted the tumor was seen to extend anteriorly, passing about the cord in a saddle fashion and becoming visible on the right of the cord as it was retracted to the left. It had also infiltrated the marrow of the vertebræ, and through one intervertebral disc it had penetrated the left pleural cavity. Dura not involved. This mass was excised. Pathological diagnosis on this tumor mass: "malignant leiomyoma."

Patient gradually regained the use of her legs and control of sphincters. Coley's serum administered. On May 4, 1915, patient was discharged, relieved, with a diagnosis of "sarcoma of the spinal cord with metastases."

After her discharge she developed numerous metastases, producing various symptoms which confined her to bed. On November 12, 1915, she died, and the body came to autopsy.

Autopsy 3 hours post mortem. — The body is that of a large, well-developed, well-nourished, white female. The scalp presents numerous elevated nodules 1 to 3 centimeters in diameter, firm, and freely movable beneath the skin. The left eye protrudes 2 to 3 centimeters from its normal position. Its cornea is steamy and its pupil, oval in shape, measures 2 x 4 millimeters.

Freely movable subcutaneous nodules are present in the right breast, the abdominal wall, the right infrascapular region, and the right lumbar region, varying from 2 to 7 centimeters in diameter. The skin covering the last-mentioned nodule presents a marked bluish discoloration.

There is an old mid-line suprapubic surgical scar, 5 centimeters in length, and another posteriorly in the mid-cervical region, two centimeters in length. A large decubitus is noted over each trochanter.

Abdomen: On section of the panniculus (4 centimeters in thickness) near the umbilicus there is a freely movable tumor nodule embedded in the fat; another is seen in the substernal notch. Small tumor metastases occur on each side of the pelvic and lumbar spinal column and a large mass almost fills the pelvis. The mesenteric lymph-nodes are negative. The appendix has been removed.

Pleural cavities: The left side shows extensive involvement of the first three ribs with tumor, which is apparently extending from the spinal column. The costal periosteum is raised by numerous smooth, rounded nodules, which involve chiefly the thoracic side of the ribs. The right cavity presents a few slender adhesions along the lower border of the lung. Palpation reveals a tumor mass lying directly beneath the surface of the lung at this point.

Heart: Aortic stenosis and microscopic focus of tumor cells in the myocardium.

Lungs: Not remarkable except for the presence of tumor metastases. Scattered throughout the entire substance of both lungs are numerous

sharply-circumscribed, firm, yellowish-white nodules, ranging from .5 to 2 centimeters in diameter, which shell out of the tissue with great readiness.

Liver: The organ is large and considerably above normal weight. Scattered over its generally smooth yellowish-brown surface are very numerous raised tumor nodules, varying from .2 to 10 centimeters in diameter. On section a similar appearance is noted; the tumors are sharply circumscribed and bulging.

Spleen: Negative.

Kidneys: Numerous tumor nodules, chiefly confined to the cortex, and measuring from .1 to 1.5 centimeters in diameter.

Adrenals: Negative.

Pancreas: Tumor masses, the largest 2.5 centimeters in diameter, located near the head of the organ in the region of the duct of Wirsung.

Gastrointestinal tract: Negative.

Pelvis and contents: A previous supravaginal amputation has been performed, leaving a smooth pelvic floor covered by peritoneum and apparently free from tumor growth. On either side, in the position of the broad ligaments, are large nodular masses, the smaller, on the left, measuring 3 x 5 centimeters; and the larger on the right, with a constriction between its middle and outer thirds, measuring 4 x 5 x 10 centimeters. These masses are roughly spherical in shape and nearly fill the cavity of the pelvis.

Aorta: Marked sclerosis with calcification and erosions. Along its course for about 5 centimeters above the bifurcation are several small tumor masses.

Brain: Granular ependymitis involving the left lateral ventricle only.

Middle fossa of skull and right orbit: Bulging backward into the fossa from the orbit is a subdural tumor mass which has eroded the skull to some extent and is causing pressure on the optic nerve, chiasm, and adjacent structures. Following the mass forward into the orbit it is found to occupy almost the entire cavity and to have displaced the eyeball forward.

Spinal cord: Beneath the previously-mentioned scar in the cervical region, the spinous processes and laminæ have been removed from the 7th cervical to the 3d dorsal vertebra. The site of operation is overlaid with dense scar tissue which is infiltrated with tumor. This tumor tissue is found to be a direct continuation of that already described on the ribs of the left pleural cavity. Opening the vertebral canal reveals a large growth in the 5th or 6th cervical region, measuring 3 x 5 centimeters in diameter, which is adherent to the dura and which is compressing the cord to about one-half of its normal anteroposterior diameter. Directly above this the dural canal is invaded by an annular growth for about 2 centimeters of its course. No invasion of the dura or of its contained structures is observed. Above and below this point no gross lesion can be seen. Histologically the cord shows sclerosis of the posterior columns

in the cervical regions and of the crossed pyramidal tracts in the dorsal and lumbar regions.

Tumor: The various nodules all present the same general characteristics, namely, circumscribed masses of tissue which are pale pinkish-yellow in color, firm in consistence, and generally smooth, although in one or two instances (*e.g.*, pelvic and pancreatic masses), the surface is nodular. The size varies greatly; many nodules are less than 1 centimeter in diameter, while in the liver masses as large as 10 centimeters in diameter are seen. Although the tissue is firm the finger nail may readily be pushed through its substance. Section shows a smooth yellowish surface with an opalescent cast. The arrangement of the fibers in ramifying bundles and whorls is pronounced. All the nodules are very loosely imbedded and on section they bulge from the surrounding tissue.

Histology. — The nodules removed surgically from the spinal cord and from the skin were fixed immediately in Zenker's fluid and in these nodules alone the minute fibrils of the cells could be demonstrated. Although the autopsy was performed only three hours after death, these fibrils had largely disappeared from the tumor cells.

The different nodules are all definitely surrounded by a connective tissue capsule, prolongations of which extend into the substance of the tumor and divide it into lobules. This connective tissue forms a framework which carries numerous blood vessels. At no point does the tumor invade its capsule or the surrounding tissues, but rather pushes aside and compresses them.

The tumor cells, in longitudinal section, are large and oval in shape. The cytoplasm is finely granular, deeply acidophilic, and is sharply limited by numerous myoglia fibrils. The nuclei are of the type usually described as "rod-shaped," large and oval with blunt, rounded ends. In places these cells change gradually to a type more rounded in shape, larger, and often containing multiple nuclei. Mitotic figures are frequent, as many as three or four to an oil immersion field. In cross section each cell presents a small round nucleus, surrounded by a clear zone which is in turn surrounded by a definite membrane in which may be seen sections of myoglia fibrils.

The tissue from the cervical dura shows a section of a small vein, the lumen of which is almost filled with a mass of rapidly-growing tumor cells. This vein is located in the connective tissue at some distance from the main tumor mass, which apparently rules out the possibility of artefact.

Examination of a celloidin section from the uterine tumor removed at the first operation reveals very frequent mitotic figures in smooth muscle cells, two to five in a high dry field. Although tissue so preserved is not suitable for the demonstration of myoglia fibrils, the presence of

numerous mitotic figures in this nodule in conjunction with the findings in the nodules in other organs would very obviously point to this tumor as the primary source of the growth.

Discussion. — As before mentioned, any attempt at a synopsis of the literature of metastatic leiomyosarcoma is unprofitable, owing to the lack of a standard of comparison. Many cases of so-called uterine sarcoma have been reported. In some instances the authors have been certain that the tumor cells were of true smooth muscle type; in others, the tumors were thought to result from a metaplasia of muscle cells to "sarcoma cells;" still another group was explained as arising from the connective tissue stroma of the primary growth. The first and last groups offer no difficulties, but metaplasia of cells from one embryonic type to another seems hard to accept. In view of the relative frequency of histologically malignant leiomyoma, which remains localized, it would seem likely that many of the reported metastatic cases are of smooth muscle origin. Moreover, in support of this supposition may be urged the fact that smooth muscle tumors are most frequent in the fundus uteri, while connective tissue tumors occur in the cervix. Most of the cases reported have shown primary growths in the fundus.

As an absolute criterion for the recognition of smooth muscle, Mallory accepts the "Grenzfibrillen" or "myoglia fibrils," first observed and described by Heidenhain. These fibrils are found intact only in tissue fixed immediately in Zenker's fluid, and are best shown by Mallory's phosphotungstic acid hematoxylin. They are present in all smooth muscle cells, and although they are not differentiated by color reactions from the fibroglia fibrils of the fibroblast, yet they have certain definite morphological characteristics, namely, their situation in the limiting membrane of the smooth muscle cells and their fusion at the ends of the long spindle cells to form coarse fibrils. In cross-section about a dozen myoglia fibrils are seen lying in the limiting membrane of the cell. The presence of myoglia fibrils will, of course, be suggested by the general appearance of a cell having the features

usually ascribed to smooth muscle; namely, rod-shaped nucleus, spindle-shaped cells with definite outline and staining deeply with acid dyes.

The fibroglia fibrils of the fibroblast, from which myoglia fibrils must be differentiated, also lie in the limiting membrane of their respective cells in the region of the nucleus, but are always fine and spread out in a more or less fan shape at some distance from the cell body. The adult fibroblast also produces another intercellular substance, collagen, which is laid down in large bundles of fine fibrils. The other characteristics distinguishing this cell from the smooth muscle cell are its flattened oval nucleus, its lightly-stained acidophilic cytoplasm, which sends out two or more processes and its oval shape in cross-section.

Most of the authors have failed to describe the type cell of their tumors with reference to its fibrils. It would seem that an essential characteristic has been hitherto neglected. From their general descriptions it would seem that the tumors described by Krische, v. Beesten-Minkowski, Langerhans, Schlagenhaufer, Hoevels, West, v. Mastńy, v. Kahlden, and some of Gessner's cases were actual smooth muscle tumors, which metastasized from primary growths in the uterus. It is very likely that other tumors might also be so classified, but the data are often too scanty.

As to the proper terminology for such tumors, it seems advisable to adopt Ribbert's designation of sarcoma for all tumors not of epiblastic origin. The type-cell, here the leiomyoblast, must also be included. Consequently, Mallory has adopted the name leiomyosarcoma.

The question of malignancy and tendency to metastasis in such tumors is admirably covered by Aschoff, who says: "These are histologically benign tumors, which occasionally rupture into vessels. By a remarkable resistance in the blood stream, their cells succeed in settling and proliferating in the lungs. Whether such tumors should be considered as malignant is not worthy of dispute, for all gradations exist between benign and malignant tumors. In some cases the malignant character of the neoplasm in the uterus and its destructive

action on the surrounding tissues is very marked. The tumor cells not only displace but infiltrate the myometrium and destroy its muscle cells."

In the case above described, all the requirements of metastatic leiomyosarcoma have been fulfilled; namely, a rapidly-growing primary tumor has been removed from the uterus; the tumor cells are seen actually growing in a vein; multiple metastases are found in the viscera, showing typical smooth muscle cells.

[In conclusion, I wish to express my sincere thanks to the many physicians of Boston who had seen this patient prior to her death, and who afterward spared no pains in obtaining data for me. Especially I would thank Dr. A. A. Cushing, who obtained permission for autopsy; Dr. C. A. Hare, whose case the patient originally was; Drs. J. H. Wright and T. Leary for fresh surgical material, and Dr. F. B. Mallory for his many helpful suggestions in the preparation of this paper and for the photomicrographs.]

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DESCRIPTION OF PLATES I. AND II.

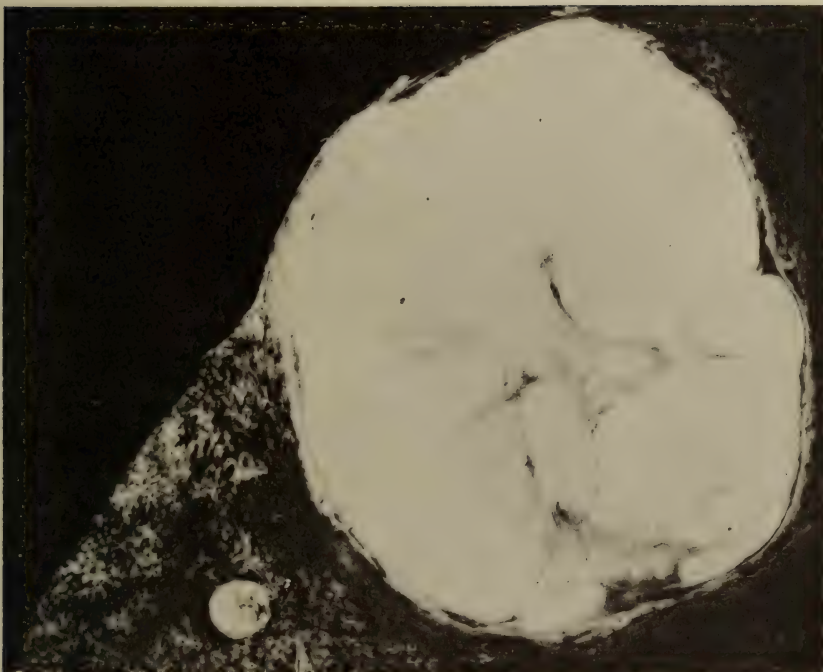
The nuclei and myoglia fibrils in Figs. 2, 3, and 4 were stained with phosphotungstic acid hematoxylin.

PLATE I., FIG. 1. — Section of two metastatic nodules of leiomyosarcoma in the liver.

FIG. 2. — Edge of nodule of a metastasis of the leiomyosarcoma in the skin showing the marked contrast between the smooth muscle cells of the tumor and the fibroblasts of the connective tissue capsule. x 250.

PLATE II., FIG. 3. — Same section as Fig. 2 showing rapidly-growing area in which four mitotic figures are plainly visible. x 250.

FIG. 4. — Same section. Two mitotic figures and one leucocyte present among the smooth muscle cells. x 1,000.



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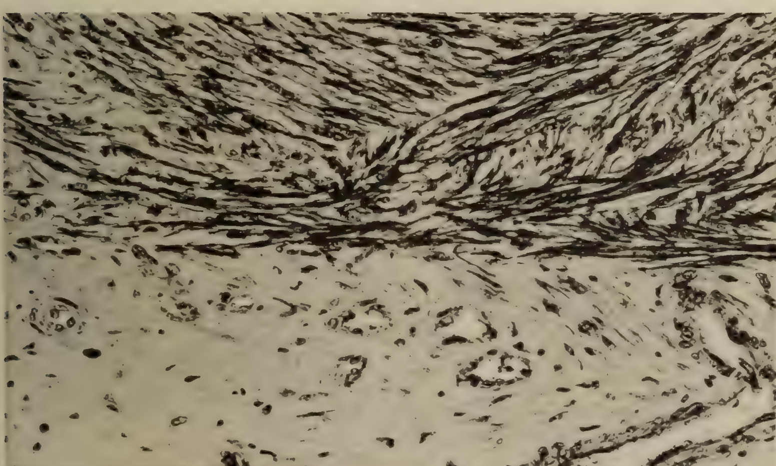

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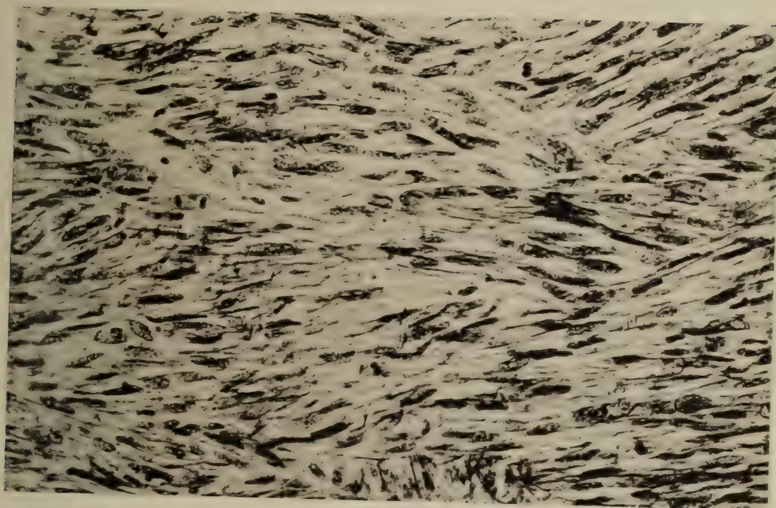
Gardner. Leiomyosarcoma.



3

4

Gardner.

Leiomyosarcoma.

THE INFLUENCE OF SERUM UPON THE STAINING OF BACTERIA IN SUSPENSIONS.*

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The point of origin of these experiments was the observation that serum added to a suspension of bacteria in dilute stains interferes with or inhibits the staining of the organisms. Since the bacteria were not stained by acid stains — eosin and acid fuchsin — this inhibitory action was noted only in the case of basic stains, of which we used neutral red, methylene blue, gentian violet, methyl green, and Bismark brown.

The stains were usually used in dilutions of 1 to 20,000; however, methyl green was used in a dilution of 1 to 10,000, and Bismark brown in a dilution of 1 to 4,000.

Serum was as a rule used in a dilution of 1 to 25, and the experiments when not otherwise stated were carried out with this dilution.

The various mixtures were made in small test-tubes; as a rule the serum was added first in a dilution half that which we actually wished to use; sufficient .85 per cent sodium chloride solution was added to make the total quantity of fluid, including the stain up to one cubic centimeter, and finally the stain was added. In the majority of the experiments we used Bacillus coli, but we also tested the action of serum on other Gram-negative and several Gram-positive bacteria, and in all cases the action was the same. A few drops of a homogeneous suspension of washed bacteria added to the one cubic centimeter of the mixtures in the tubes, made a faintly-cloudy suspension, in which the bacteria were present in sufficiently large numbers to enable us to study carefully the staining of the organisms. The bacteria were usually found singly or in pairs in these suspensions, unless they had been exposed to the action of some

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agglutinative substances, such as immune serum, certain stains, or acids.

The staining of the bacteria was always determined microscopically. Since the mixtures were usually allowed to stand over night — from eighteen to twenty-four hours — at 37.5° C., it was necessary to prevent the growth both of possible contaminations or of the organisms which were being examined; for this purpose we added a small quantity of thymol.

The influence of serum upon staining of bacteria. — As we have stated above, the serum was usually used in a dilution of 1 to 25, and we shall first consider its effects in this dilution. Since acid stains were not taken up by the bacteria in suspension and since these stains became active only after acid had been added, it is evident that we need not consider the inhibitory action of serum in such mixtures of serum and acid stains. Neutral red, which colored the bacteria fairly deeply, completely lost its power to stain the organisms when serum was added. As was to be expected when this stain was mixed with a slightly alkaline fluid such as serum, the red color was lost and the mixture showed an opalescent amber-yellow color. In a few instances the bacteria took the stain very slightly; in such cases they were not stained red, but very pale yellow to brownish.

Of all of the stains methylene blue was least affected by the addition of serum; in most cases its staining power was slightly inhibited, but in a few cases the bacteria were as well stained as with the staining fluid alone. In those cases in which the action of the serum was evident, the bacteria were simply stained a paler bluish shade than the bacteria in the control tubes. The color of the fluid was not changed by the addition of serum, but showed a slight opalescence.

Gentian violet, which stains the bacteria deeply, was very markedly affected by the serum, and it was in exceptional cases that the bacteria were stained at all by this substance when mixed with serum. Occasionally we did find instances

in which the organisms were stained, and it is interesting to note that it was the single organisms and smaller groups of two to four organisms which were stained, while the larger clumps were faintly colored, if at all.

Bismark brown was interfered with quite markedly by the serum, although the power of staining was not as a rule completely inhibited. We usually found that the addition of serum had a marked inhibitory action, and the bacteria were stained a pale yellow, instead of the rather deep brown which the organisms usually took. Only exceptionally was this action not evident.

Methyl green, which like neutral red was sensitive to alkalinity, did not show as marked an effect of the alkalinity of the serum; the former was never as markedly reduced as was neutral red. With some of the sera used the color of the methyl green solution was practically the same as in the mixture without the serum. The bacteria were very poorly stained, or even not at all; they were never as well stained as were the control bacteria.

We see, therefore, that the addition of serum to the mixture of bacteria and stains had in every case interfered with the action of the stains, interfering only slightly with methylene blue, and markedly with the four other basic stains.

Dilution of the serum. — The serum was at first used in concentrations of 1 to 25 or 1 to 50, and it appeared that in the higher dilution the action was a trifle less evident, we therefore carried out a number of experiments in which the quantity of serum was varied, while the quantities of the other substances were constant. The inhibitory action of the serum was compared when used in dilutions of 1 to 25, 1 to 50, 1 to 100, 1 to 200, and 1 to 500.

When the serum was used in the higher dilutions it was evident that the inhibitory effect was gradually lost. When used in a dilution of 1 to 500, methylene blue, gentian violet, methyl green and Bismark brown stained the bacteria as well as in the control experiments; neutral red alone was

slightly affected by this dilution of serum, but only in a slight degree.

The addition of serum in dilutions of 1 to 100 had but little effect on the staining by methylene blue or gentian violet, and methyl green and Bismark brown were scarcely affected by the addition of serum in quantities of 1 to 200.

We see, therefore, that with a gradually increasing dilution of the serum its effect becomes less and less evident, until when present in a dilution of 1 to 500, the effect upon most of the stains is either entirely or almost entirely lost.

Influence of varying dilutions of stain. — We have noted above that the more concentrated solution of the serum gave the most marked inhibitory effect, it was further necessary to determine that concentration of the stains which would give a sufficiently deep coloring of the bacteria in the control-tubes and yet show distinct evidence of the action of the serum. For this purpose we tested the action of a number of dilutions of the various stains used in the experiments.

In all cases when we varied the concentration of the stains we used the serum in a dilution of 1 to 25.

The lowest dilution used with most of the stains was one of 1 to 5,000, and we varied the dilutions from this up through 1 to 10,000, 1 to 15,000, 1 to 20,000, 1 to 30,000, and 1 to 50,000. With methyl green we used the following dilutions: 1 to 5,000, 1 to 7,500, 1 to 10,000, 1 to 15,000, and 1 to 25,000; with Bismark brown 1 to 2,000, 1 to 3,000, 1 to 4,000, 1 to 6,000, and 1 to 10,000.

We found that when the stronger stains were used the action of the serum was less marked, indeed when methylene blue was used in a dilution of 1 to 15,000 and gentian violet in 1 to 10,000 the bacteria mixed with the serum stained almost if not quite as well as the controls. In fact, in none of the dilutions used was the staining by methylene blue completely inhibited.

Bismark brown, methyl green, and neutral red all stained the bacteria less deeply when serum had been added to the suspensions and this action was noted with all concentrations of the various stains.

While methylene blue was never completely inhibited by the serum it was found that staining by gentian violet and neutral red was completely inhibited when these stains were used in dilutions of 1 to 20,000, and methyl green and Bismark brown were completely or almost completely inhibited when used in dilutions of 1 to 10,000 and 1 to 4,000 respectively. These two latter stains could not be used in lower dilutions, as they then stained even the control bacteria but faintly.

We note, therefore, that in all cases the greater concentrations of the stain appear to overcome at least to a slight degree the inhibiting effect of the serum — most markedly in the case of the gentian violet and methylene blue, but in the majority of the stains even with the strongest concentrations used, the inhibiting action of the serum although weakened still persists.

The influence of various sera and various constituents of serum. — The experiments so far mentioned were all carried out with rabbit serum. It appeared to be of interest to determine whether serum of other animals would show a similar inhibitory action. For this purpose we used guinea-pig serum, dog serum, and human serum, and found that the action of these other sera was identical with that of the rabbit serum.

We used not only fresh serum, but also serum which had been kept (in the ice-box) for some time — in some cases as long as four weeks; serum which was preserved with .4 per cent phenol; normal serum as well as anti-colon or anti-typhoid serum, and serum which contained anti-bodies in no way related to the bacteria used in the experiments — such as precipitins against egg white. These sera all acted in a similar manner in inhibiting the staining.

The heating of serum to 56° C. for thirty minutes, or to 100° C. in a diluted state for thirty minutes, or to 120° C. (in dilution) for fifteen minutes, had no effect upon the inhibiting power of the serum.

We precipitated the globulins of the serum by two

methods, either by passing CO_2 through the serum for some time, or by diluting the serum with distilled water; the precipitated globulins were separated by centrifugalizing, and the supernatant fluid was then added to the mixture of the bacteria and the stains. We furthermore washed the precipitated globulin, then took it up in .85 per cent sodium chloride solution, corresponding in quantity to the fluid in which it had been originally dissolved and tested the inhibitory action of this globulin solution.

The residue of the serum, freed from the globulins, acted in the same manner as did the normal serum. The globulins, however, did not prevent the staining of the bacteria; even when the concentration of the globulins was but slightly less than in undiluted serum, there was no inhibiting action evident.

In a similar manner we determined the effect of at least a partial removal of the lipoids from the serum; we shook the serum with chloroform — using two volumes of this latter to one volume of serum, shaking for ten minutes, and finally separated by centrifugalizing. The serum, freed of the lipoids in this manner, shows the same inhibitory action as does the whole serum, while the lipoids, when redissolved and made up to their original volume with salt solution, had no inhibitory effect.

It seems, therefore, that the action of the serum in inhibiting the staining of the bacteria is in all probability dependent upon the serum albumin, rather than upon any other constituent.

The influence of acid and alkali. — Since the serum albumin is alkaline in reaction, and since the solution of the serum and the stain when mixed, showed with neutral red an alkaline reaction, it seemed possible that the effect of the serum was dependent upon the reaction of the mixture, and we therefore investigated the influence of the addition of acid and alkali upon the staining of the bacteria.

We used various normal solutions of either hydrochloric acid or sodium hydroxide.

When the acid was used in N/250 solution, there was slight evidence of inhibition on neutral red, methylene blue, and gentian violet, never, however, as marked as the inhibition by serum, excepting, possibly, in the case of methylene blue, where the degree of inhibition was about the same. A similar solution of alkali interfered very markedly with the staining of the bacteria by neutral red — quite as markedly as did the serum — and also with the staining by methyl green, but relatively not to as marked a degree as with neutral red, and also probably to a less degree than did the serum. The other stains were not interfered with by the addition of alkali of this concentration.

A solution of N/500 acid inhibited to a very slight degree the staining of the bacteria by methylene blue, but had no effect upon any of the other stains, unless it was to aid the staining of the bacteria by neutral red and methyl green. A N/500 alkali solution had an action similar to the N/250 alkali solution, but did not interfere to as great an extent. There may also have been some slight inhibition of the staining with Bismark brown by the N/500 alkali, but it was only very slight; the color of the alkaline Bismark-brown solution was not as deep brown as the stain alone, but tended rather toward a yellowish tinge.

Acid in either N/1,000 or N/2,000 solution had not any effect upon the staining, unless to interfere slightly with methylene blue in the higher concentration. There was also a slightly beneficial effect of the weak acid in the case of the neutral red and methyl green.

Both N/1,000 and N/2,000 alkali interfered with neutral red and methyl green, but to a less degree than the higher concentrations; we could even note a slight difference between the action of these two concentrations, in that the lower concentration had less inhibitory action. Bismark brown was also very slightly interfered with by the higher dilutions of the alkali.

We note, therefore, that the hydrochloric acid interferes slightly with methylene blue, but does not have any action upon the other stains. Sodium hydroxide, however, does

inhibit to a very marked degree the staining with neutral red, even in the lowest dilutions used, but shows a gradually diminishing action as the concentration of the alkali is reduced; methyl green is affected similarly but less markedly. Bismark brown is slightly affected by the alkali. In no case excepting N/250 alkali and neutral red, and N/250 acid and methylene blue was the action of the acid or the alkali as marked as the action of the serum, and upon gentian violet the action of the reagents was practically, if not actually, nothing.

The influence of addition of acid to the serum. — While the results obtained with the addition of acids and alkali to the suspension of the bacteria in the stains would not tend to support the theory that the inhibition of the staining was due to the alkalinity of the serum, it still seemed worth while to determine what influence the addition of acid to the serum might have upon the inhibiting action of the serum. We tested the same concentrations as were used in the experiments mentioned above. We found that when we added N/2,000 acid to the serum the solution was usually nearly neutral, using neutral red as an indicator; a drop of diluted serum would give an alkaline reaction and the yellow coloration of the fluid. (Thus we found that .5 cubic centimeter of N/1,000 hydrochloric acid added to .5 cubic centimeter of 1 to 12.5 dilution of serum gave a solution which was approximately neutral; of course the degree of alkalinity of the individual sera varied slightly about this point.)

The addition of acid to the mixture of stain and serum did not interfere with the inhibition in the case of neutral red or methylene blue, but did interfere to a slight degree with the inhibition by the serum of staining of the bacteria by gentian violet, methyl green, and Bismark brown.

It is also interesting to note that when acid stains such as eosin and fuchsin were used with the mixture of acid and serum (when the acid was present in sufficient quantity so that the bacteria did take these acid stains) there was evident no inhibitory action of the serum; the bacteria were as well stained in this mixture as when no serum was present.

The influence of addition of alkali to the serum. — We have seen that the alkali alone may interfere with the staining of the bacteria in the cases of neutral red and methyl green, and we therefore wished to determine whether an increase of the alkalinity of the serum might further add to the effect of inhibition. Sodium hydroxide solution was added to the serum in quantities similar to those used with the hydrochloric acid solutions. The results were identical with all concentrations of the alkali.

Neutral red did not stain the bacteria at all, but the resulting inhibition was no greater than when serum was used alone.

Methyl green, gentian violet, and Bismark brown were inhibited to as great a degree by the serum alone as by the mixture of serum and alkali, and the degree of inhibition was greater than with alkali alone.

Only in the case of methylene blue did we find a greater effect when serum and alkali were combined, than when either serum or alkali were used alone, and here it seemed that the greater quantities of alkali interfered more than did the smaller quantities.

Addition of the alkali to the serum seemed therefore to have no influence on the action of the serum in the case of any of the stains, except methylene blue.

Addition of serum or alkali after the stain. — Were the action of the serum due to the alkalinity of the fluid the full effect of the inhibition should be evident even when the serum was added after the bacteria had been thoroughly stained with the various stains. In order to determine whether this effect was evident we placed the bacteria in contact with the stains over night and then, at periods varying from thirty minutes to two hours before the examinations were made, we added serum to the mixture. The time elapsing between the addition of the serum and the examination had no influence upon the results. We added in each case a sufficient quantity of serum to make the mixture correspond to a 1 to 25 solution of serum.

The serum when added after the bacteria had taken the stain did inhibit to a certain degree the staining with neutral red, methyl green, and Bismark brown, but had no action upon gentian violet or methylene blue. As a rule the bacteria were better stained by neutral red and methyl green when serum was added after the stain, than when stain and serum acted upon the bacteria simultaneously; Bismark brown was never as markedly inhibited in this series as when serum and stain acted simultaneously.

We also carried out experiments in which we added alkali to the suspension of the bacteria in the stains in the same manner as we had added the serum. We used N/1,000 and N/2,000 sodium hydroxide solutions, and noted practically no difference in the effect of these two concentrations.

When the alkali was added to the mixtures after the stains had been in contact with the bacteria for some time, the effect was the same as when the serum and stain were added simultaneously; neutral red and methlyl green were largely or completely inhibited and the other stains were not affected. As in the case when alkali was added at the same time as the stain, methyl green was relatively less interfered with by the alkali than neutral red.

We again note that while we cannot draw from these results the definite conclusion that the action of the serum may not, at least in part, be due to alkalinity, we can certainly state that the inhibition is not entirely due to the reaction of the serum, since in this series the serum does not inhibit to the same degree as does the alkali. Serum did not produce the same degree of interference when added after the stain as when the stain and serum are added at the same time, while the alkali on the other hand showed the same degree of inhibition when added before or after the stain. When we compare the staining of the bacteria when serum was added after the stain, with the staining of the bacteria when we added N/2,000 alkali after the stain, we note that the staining with neutral red was more markedly inhibited in the latter case; however, the organisms

were stained equally well with methyl green and Bismark brown in both cases.

Influence of the exposure of the bacteria to serum before the exposure to the stains. — Since it appears from the above experiments that the inhibition of staining of the bacteria by the serum was certainly not due to alkalinity alone, it became necessary to seek for the explanation of the action through some other mechanism.

It seemed quite possible that the action was due to a thin layer of serum forming about the bacteria, an adsorption of the serum by the bacteria. If this were the case it might be possible to demonstrate a similar inhibitory action of the serum, when the bacteria were allowed to remain in contact with the serum for some time — over night — before they were added to the stain. It is important that we bear in mind the fact that we actually added sufficient serum to the suspension of the bacteria in the stain to make a dilution of somewhat less than 1 to 200, a dilution which does not interfere to any very great degree with the staining of the bacteria.

It is sufficient to state that the action of the serum in inhibiting the staining was slightly evident in all cases; methylene blue and methyl green being only very slightly interfered with, gentian violet and Bismark brown being irregularly and slightly inhibited, and even neutral red was only slightly interfered with. This action is practically the same as that of a similar dilution of serum — namely of 1 to 200 — and we have been able to obtain no definite evidence that the action of the serum is dependent upon a protective colloid action.

The influence of egg white and ovalbumin. — We considered the possibility that the inhibitory action might be specific for serum, and determined therefore to test the action of similar substances.

We repeated a series of experiments with either ovalbumin or egg white (using chiefly the latter), similar to

those which we had performed with serum. The ovalbumin was made in a stock solution of about three per cent and was diluted in our experiments, so that we used a 1 to 25 dilution of this stock; egg white we used chiefly in a 1 to 25 dilution. We found that our results with the two substances were identical, and therefore used the latter in most of our experiments.

We may state that on the whole the action of egg white was very similar to that of the serum, differing not in character but only in degree; the action of the egg white appears to be slightly less marked than that of the serum. Egg white in a dilution of 1 to 25 interferes with all the stains to a quite marked degree — least with Bismark brown and methylene blue. When the egg white was used in higher dilutions the action was less marked, especially in the cases of gentian violet, methyl green, and Bismark brown. In a dilution of 1 to 100 the action on neutral red showed a marked diminution and the bacteria were fairly well stained. It is possible that the action of the egg white was not as marked or as clear cut as that of the serum owing to the formation of precipitate when the stain and egg white were added.

(We find that practically all the basic stains produced a precipitate with egg albumin. This action was somewhat irregular, and was possibly least marked with gentian violet. There seems to be little or no definite relationship between the formation of precipitate and inhibition of staining or varying degrees of this inhibition. The addition of either acid or alkali to the mixture of egg white and stain had little or no influence on the formation of the precipitate.)

The addition of acid does interfere to a slight degree with the inhibition of the staining of the bacteria by gentian violet and Bismark brown produced by the egg white, which is consistent with the less marked action of the egg white as compared to the serum. We further note that the addition of acid to egg white in a similar manner as the addition of acid to serum, interferes with the inhibitory action in the cases of gentian violet and Bismark brown; in the case of the serum the acid also prevents the action in the case of

methyl green, but does not in experiments with egg white. Thus the action of addition of acid to these protein solutions has a very similar though not identical action in both cases.

When alkali was added to the egg white the action of inhibition was evident in neutral red, methyl green, and Bismark brown, but the alkali did interfere to some degree with the inhibition of staining in the case of gentian violet and methylene blue. This action of the alkali was most evident when the higher concentrations were used. It was evident here as in the case of the serum that the action of the egg white in inhibiting the staining was not due entirely to the alkalinity of the solution, as the inhibition by the mixture of the egg white and alkali was greater than that by the alkali alone, but not more marked than by the egg white alone.

Here, as in the experiments with acid, we find certain differences between the action of the addition of alkali to serum and to egg white; while the addition of alkali to serum does not interfere with the inhibitory action of the serum in any cases, it does interfere with the action of egg white, in the case of methylene blue, and gentian violet. We find, however, here no evidence for the belief that the action of the egg white in inhibiting the staining is due to alkalinity, since the increase of alkalinity has not increased the inhibition of the staining.

In the experiments carried out with the addition of either acid or alkali to the egg white, we found that the egg white was precipitated in very much the same manner as when the egg white was added to the stains alone, and it is quite possible that this precipitation may account in part for the divergence in results obtained with serum and egg white.

The influence of casein. — We used a stock casein solution of .1 per cent and diluted this either four or ten times with sodium chloride solution. This solution proved to be nearly neutral or slightly acid; the solution when mixed with neutral red did not change the color of the stain.

The casein in either dilution did not interfere with any of the stains with the possible exception of Bismark brown, and

this stain was inhibited to only a very slight degree. When either acid or alkali was added to the casein in a concentration of N/1,000 there was no change in the action, the only stain affected was again Bismark brown which was only slightly inhibited.

Casein has therefore no inhibiting action on the staining of bacteria by the basic stains, except in the case of Bismark brown.

The influence of gelatin and glucose. — The substances other than acids or alkalies which we have so far noted as inhibiting the staining of the bacteria, have all been alkaline in reaction. We now tested the action of two substances, both giving an acid reaction, namely, glucose and gelatin.

Gelatin was used in a three-per-cent-solution — that is when mixed with the stain and bacteria it was present in three parts in one hundred. The action of gelatin was neither marked nor constant, but in practically all cases there was a slight inhibition. We find that when we add to the gelatin sufficient alkali to correspond to a N/250 solution of sodium hydroxide, which was, however, not sufficient to completely neutralize the solution, the inhibitory action of the gelatin is largely destroyed. However, we also found that when we made the gelatin solution more acid, adding N/250 acid — the gelatin again loses its inhibitory action.

While we have seen previously that the stronger acid solutions may interfere with the staining of the bacteria by neutral red, methylene blue, and gentian violet, we can state that the action of the acid is not as marked as the action of the gelatin; that the action is not due to the acid alone appears from the fact that the addition of acid to the gelatin destroys the inhibitory action of this substance. It seems most likely that the explanation of this phenomena is that the gelatin acts as a protective colloid, and that the changes of the reaction of the media resulting from the addition of acid or alkali produce such changes in surface tension, that the gelatin is no longer adsorbed by the bacteria.

Glucose was used in a five-per-cent-solution; the action

was very slight; neutral red and methylene blue were inhibited in practically the same degree as were the same stains by the N/250 solution of acid; the other stains were still active as in the control-tubes. Addition of N/250 acid did not influence the action of the glucose, and addition of N/250 alkali, which was sufficient to make the solution alkaline, interfered with the staining by neutral red, methyl green, and Bismark brown, in the same manner as did a weak alkaline solution alone. We noted that the glucose solution aided the staining of the bacteria by methyl green, and with the combination of this substance and methyl green we obtained a more brilliant staining of the bacteria than by any other method tried.

Glucose, therefore, we note, has no action in inhibiting the staining of bacteria, except that which we might expect from the reaction of the solution, and this corresponds fairly closely with the results which we have obtained with the acids and alkalies alone.

Discussion. — We find little or nothing in the literature concerning the inhibition of staining by serum or similar substances. The observations of Mosso[1] and Plato[2] are not analogous to the results reported above.

Fischer[3] has noted an action of albumins in preventing the staining of tissues with methylene blue and methyl green. He states that certain fixatives, such as platinum chloride, Hermann's solution, tannin, osmic acid, mixtures of iodine and alcohol and albumins, will totally prevent the action of the stains. This preventive action of these substances he believes to be due to the saturation of the materials with the fixatives. He further found that albumoses, precipitated by platinum chloride, when soaked in five-per-cent glycocol, showed slight evidence of inhibition; in the case of precipitated nucleinic acid, the inhibition by glycocol was more marked. Fischer used in these latter cases gentian violet and methyl green, and also methylene blue, which was, however, not inhibited.

The experiments of the three investigators mentioned were

carried out with the idea of determining the nature of the action of stains. We do not, however, wish to enter into a discussion of the theory of staining. We are concerned chiefly with the explanation of the phenomena of the inhibition of the action of the stains in the presence of serum.

We might explain this action of serum in four different ways: First, that the inhibition is the result of the change of reaction of the fluid; secondly, that the phenomenon is due to reductive changes in the stains; thirdly, that it is due to increased solubility of the stain in the diluent fluid; fourth, that the inhibition of staining is due to a protective colloid action, and that the action be explained upon a basis of physical rather than chemical phenomena.

As regards the first theory, we have shown that while serum is alkaline, the neutralization of this alkalinity does not destroy the power of the serum to prevent staining by the basic stains, nor does an increase in the alkalinity increase the action of the serum. Furthermore, we found that certain acid substances, such as gelatin, will also prevent the staining of the bacteria, although not to as marked a degree as the alkaline serum. Again the action of the serum cannot be duplicated by the use of a weak alkaline solution, although in the case of neutral red and methyl green, and very slightly in the case of Bismark brown, we note a similar action. It is true that we find that certain acid substances such as serum globulins, casein, glucose, do not prevent the staining as does the whole serum or the serum from which the globulins have been extracted, and it is further true that other alkaline substances, as egg white or egg albumin, do not interfere with the staining in the same manner as does serum; nevertheless the evidence mentioned above is such that we must believe that the inhibitory action of the serum is not due to the alkalinity of the fluid. We cannot, however, entirely exclude the reaction as a factor. Thus, although the alkalinity of the serum may play a small part in inhibiting the staining we must seek elsewhere for the principal factor.

Turning to the second suggestion, namely, that the inhibition is due to a reduction of the stains, we should first consider the fact that investigators who advanced the theory of reduction and oxidation as the principal factors in the vital staining of cells obtained results differing from ours under somewhat different conditions. They noted differences between the staining qualities of dead and living cells, or of granules living either in the ecto- or endoplasm. We also dealt in most of our experiments with living cells; and did carry out some experiments with dead bacteria no longer able to show either reductive or oxidative processes; with the dead bacteria our results were the same as with the living organisms. In the experiments in which serum was added after the bacteria had been in contact with the stain for some time, we note that it did not have anything like as marked a preventive action as when added simultaneously; had the action been due to reduction of the stain, the serum should have been equally active in the two sets of experiments. There is certainly no evidence in our experiments pointing toward reductive processes as the causes of the inhibition of the stains.

It is known that cells are not stained when the stains are dissolved in substances which will too readily hold them in solution. It cannot, however, be this factor of solubility which prevented the staining of the bacteria when in the presence of serum. Just as the basic stains produced a stained precipitate with egg white, they may produce a stained precipitate with serum albumin, if this latter substance is present in sufficient quantity. This action suggests the reverse of a greater solubility of the stain in serum and we can put aside this theory as being highly improbable.

We are inclined, therefore, to lay emphasis upon the physical explanation of this phenomena. We believe that phenomena of surface tension and adsorption are in all probability responsible for the inhibitory action of the serum. Bechhold,[4] in studying the action of protective colloids, has shown that serum has a marked action in this

regard, preventing the precipitation of a number of suspended substances such as kaolin, mastix, and hydrosols by the salts of heavy metals. He further studied the action of serum in protecting bacteria against precipitation and this protective action he considered to be due to the adsorption of the serum by the bacteria. The adsorbed serum may well in our experiments form a coating about the bacteria and thus prevent the stain from reaching the bacteria, and from acting upon them in whatsoever manner the stain does in coloring the organisms. The rate of diffusion may enter into the problem of the degree of inhibition of staining, and variations in inhibition of the individual stains may be due to the different rates of diffusion of the stains (Fischer). The addition of acid or alkali may influence not only the surface tension, and thus the adsorption of the serum by the bacteria, but also the rate of diffusion, and it is possible these two influences of the reaction may or may not work in the same direction with different concentrations of acid and alkali. That diffusion plays a part is further suggested by the fact that when we used the higher concentrations of the stains the action of the serum was not so marked, and it has been shown by Fischer that the rate of diffusion of stains increases with the concentration.

The experiments in which we exposed the bacteria to serum for some time before we added the stain, might be used as an argument against the explanation of the phenomena which we have considered as the most plausible one. Had the bacteria in these experiments failed to take the stain we would have had quite definite evidence of the correctness of our view point. We do not, however, believe that the failure to obtain this result destroys the value of this theory, since we must bear in mind that surface tension relationships are delicate phenomena and also that adsorption is a reversible reaction; we therefore cannot believe that these negative results make improbable the explanation which we believe to be the one most nearly in agreement with our results.

CONCLUSIONS.

1. Serum added to a mixture of bacteria and certain dilute basic stains, when present in one part in twenty-five, prevents the staining of the organisms. The stains used were neutral red, methylene blue, gentian violet, methyl green, and Bismark brown.

2. A similar though weaker inhibitory action can be produced by adding egg white or egg albumin instead of serum.

3. A very weak inhibitory action is exerted by three-per-cent gelatin solutions.

4. The addition of acids or alkalies to the mixture of bacteria, stain, and serum does not interfere with the inhibitory action.

5. The action of the serum diminishes with diminishing concentration of serum. An increasing concentration of the stains also tends to overcome the inhibition of the serum.

6. The inhibitory action of serum was noted when human, dog, rabbit, or guinea-pig serum was used.

7. Heating either the serum or the bacteria to 120° C. does not interfere with the inhibitory action.

8. The serum prevents the staining of both Gram-positive and Gram-negative organisms.

9. The action of the serum is probably dependent upon the serum albumin rather than any of the other substances in serum.

10. The inhibitory action of serum is not due to the alkalinity of the serum, nor to a reducing action on the stains, nor to an increase in the solubility of the stains.

11. It seems most probable that the preventive action of the serum is due to a protective colloid action.

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SOME MORPHOLOGICAL EFFECTS OF PROLONGED INANITION.*

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The opportunity to make a necropsy in the following unusual, if not unique, case came to me through the courtesy of my colleague, Professor Swain. The body was that of an American tailor who steadfastly refused all food for a period of sixty days and who died on the sixty-third day. During this long period of time nothing but water was taken, except during the last three days. For further information about the case readers are referred to Swain ('17).

Professor Swain, to whom I am indebted for the clinical data, informs me that the last weighing, taken on the fifty-sixth day, gave a net weight of 85.5 pounds. Since this was seven days before death the estimated weight of eighty pounds at the time of death, given by Swain, cannot be far from the actual. This is also indicated by the fact that the loss in weight during the eight days previous to the last weighing was ten pounds, or one and one-fourth pounds per day. Indeed, this loss would seem to make it probable that the final weight was less rather than more than eighty pounds, as assumed. The height as found on the cadaver was 1.72 meters and contrasts very strongly with the low final body weight and the normal weight of 135 pounds. The necropsy could, unfortunately, not be obtained till eighteen hours after death. There were no gross signs of post-mortem putrefactive changes, however, except slight discolorations in the inguinal regions which will be referred to later. The cadaver, to be sure, was strikingly emaciated and reminded one of what is sometimes seen in chronic cancer or tuberculous conditions. The temples, cheeks, and eyes, and the anal and para-anal regions were extremely

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sunken. The supra and infra sternal and the popliteal fossæ were very deep, and the neck was extremely thin and angular, and the abdomen scaphoid. The palmar and plantar skin lay in marked folds, but the skin over the rest of the body, although loose, lay in folds nowhere. The epidermis was decidedly dry and rough, especially on the abdomen and thighs, and looked very much as though it had been abraded before death. The skin had a peculiar mottled aspect over the abdomen, over the lower thoracic and femoral regions and on the dorsum of the trunk, and was colored a deep cherry-red over considerable areas. Professor Swain informed me that roughening and some discoloration of the skin were present over diffuse areas several days before death.

The mucous membranes were extremely pale and the conjunctivæ tinged a light yellow. This yellowish tinge was noticeable also in the skin, especially over the dorsum of the penis.

The muscles, especially the pectorals, had an intensely dark-red color, but the costal cartilages were light yellow. The peritoneal cavity contained only a few cubic centimeters of yellowish serous fluid and the pleural cavity practically none. The pericardial cavity on the other hand contained twenty-eight cubic centimeters of a light yellow fluid. There were no indications whatever of putrefaction in either of these cavities. The stomach and intestines, were only slighty filled with gas. There was no ulceration of the stomach or intestines, as reported in the case of Formal and Birney ('91). The stomach contained only a very small amount of fluid and coagulà, composed of a small amount of coffee and egg-white taken during the last days.

Only a small amount of fluid was present in the intestine, and the only other contents noticeable were small, thin, dark patches of meconium-like material on the wall of the relaxed ascending and transverse colons, and a small amount of similar material in the firmly-contracted descending colon. The iliac colon contained a few small soft scybalous masses, but the pelvic colon was practically empty. The bladder contained about 200 cubic centimeters of urine.

Appendices coli (epiploic) were completely absent, and only very small masses of omental fat remained in the intervascular areas. The periorbital fat was absolutely depleted and the eyeballs were very soft. Only a trace of the popliteal fat remained, and the palmar and plantar subcutaneous tissues had a deep cherry red, almost purplish color, and were quite firm to the touch. The characteristic gross lobulation of these tissues was preserved, however, but no fat could be recognized macroscopically in these regions. The color of these tissues was the same as that of certain areas of the skin previously referred to, and was, no doubt, responsible for the coloration of these areas.

Only traces of prevertebral fat remained. None was present in the pelvis, but some was still left near the root of the mesentery and in the mediastinum in the region of the thymus. All the viscera were very pale and flabby. The blood-vessels, including the aorta, were practically empty. There were no clots in the heart.

The gall-bladder was much distended, and the adjacent viscera were deeply bile-stained where they came in contact with it. It is unlikely, however, that all this staining was due to post-mortem changes, for I have often observed a wholly comparable condition during operation or immediately post mortem in laboratory animals.

The liver was pale and soft, but rather resistant to pressure. It weighed 914 grams.

The spleen was small and looked shrunken, for the capsule was decidedly wrinkled. It was pale and flabby and weighed only fifty-three grams.

The stomach contained about 200 cubic centimeters of fluid contents, which included some coagulum from the small amount of nutriment taken during the last three days. The mucosa looked pale and yellow. There were no ulcers, scars, or pigmented spots. It weighed 112 grams.

The pancreas, which weighed sixty-five grams, had a marked light-yellow color, similar to that of the costal cartilages, serous fluid, etc.

The renal cortex was thin, but the kidneys looked normal

otherwise. The left kidney weighed 115 grams and the right 101. The adrenals of the corresponding sides weighed twelve and thirteen grams. They were very thin.

The heart contained no clot and only a very little blood. The valves were normal and the coronary vessels were soft. It weighed 175 grams and had a markedly bifid apex, as reported previously (Meyer, '15).

There was only slight pneumonokoniosis, and both lungs had a brick-red color. The apex of the left lung was slightly adherent over an area half-centimeter square, but was otherwise normal. It weighed 324 grams. The right pleural cavity was completely obliterated and the lung could be removed only with force. This lung, with some adhering fibrous tissue, a small quantity of the intercostal musculature, and the parietal fascia, weighed 385 grams.

The bladder and prostate were normal.

The inguinal lymph nodes were barely palpable on both sides, and all except the medial nodes, which were slightly reddish, were pale. The mesenteric nodes were small and pale, but numerous. The prevertebral nodes formed a chain of soft, flat, pale bodies, and the only very red specimens were a pair of iliac nodes, one on each side. That on the right was 1 x .5 centimeters and only a few millimeters thick. That on the left was very much smaller. There was nothing noteworthy about the rest of the nodes of the entire body. Some of the right bronchial nodes were calcified. The cysterna chyli contained a little yellowish fluid.

The scalp was very adherent and was stripped with difficulty. The cranial cavity contained no more cerebro-spinal fluid than just enough to moisten the surface of the brain. The meningeal vessels contained very little blood and the dura was very pale. The brain, which weighed 1,600 grams, showed no gross changes whatever.

Aside from the emaciation, the most striking thing was the deep-red color of the muscles and the yellowish tint of the costal cartilages, the serous fluids, and pancreas. Even the hypoglossal nerves were yellow. This coloration naturally

suggested a bile stain to me, but Professor Swain found bile tests of the serous fluid negative. Since Sterzi and others have observed that the subcutaneous fat is generally more intensely stained in conditions of emaciation, one is led to think of lipochrome — whatever that may be chemically — but histo-chemical tests on fresh tissue were not tried. I have on several occasions observed a similar yellowish tinge in the serous fluids and the small amount of remaining fat in very young lambs which had died of starvation, and also in newborn colts, but am wholly at a loss to suggest the possible origin of this coloration, except from fat, as the latter is reduced or depleted.

If we assume the weight of the cadaver at the time of death to have been eighty pounds, as estimated by Professor Swain, the loss in body weight during the period of sixty-three days was 40.7 per cent. The actual weight of the individual organs must of course always remain a mere matter of conjecture. Nevertheless, the approximate weights and losses of weight can be calculated from other data. Table I., which is compiled from statistics given by Boyd, shows the actual percentage reduction in weight of the whole body of L. C. R. — the present case — and the estimated probable reduction of the various organs as determined from the average weights of the various organs, given by Boyd for paupers and lunatics, between the ages of thirty and forty years, the period in which L. C. R.'s age fell. Since Boyd's table of paupers shows a great gap between the maximum and minimum measurements, with some of the body weights as low as fifty-four pounds, it is evident that his averages are undoubtedly below the actual normal weight of the organs of L. C. R. The same criticism applies also to Boyd's averages for the insane. Consequently the actual loss in weight of the organs in L. C. R., the case reported here, was undoubtedly much larger than the calculated loss. This assumption is also borne out by the relatively greater reduction reached in the body weight of L. C. R. some time before death.

Since Boyd's measurements were made in the English they had to be reduced to the metric system for comparison. Although only one decimal is given the reductions were carefully done, but as the weights of the viscera of L. C. R. are given in the nearest gram only further reductions would have been quite needless.

TABLE I.

	L. C. R.	Insane (89–118 Cases).	Percentage.	Paupers (57–61 Cases).	Percentage.
Body weight . .	36.3 kg.	47.3 kg.	60.3 (Actual)	45.9 kg.	
Height	172 cms. (Actual)	170.7 cms.	101.4	168.9 cms.	101.8
Brain weight .	1,600 gms.	1,308.0 gms.	129.95	1,366.4 "	117.09
Right lung . .	385 "	802.8 "	47.95	807.1 "	47.83
Left lung . . .	324 "	680.8 "	47.58	688.6 "	46.90
Heart	178 "	290.7 "	61.20	332.0 "	53.60
Stomach	112 "	168.0 "	66.66	162.1 "	69.07
Liver	914 "	1,574.5 "	58.08	1,647.3 "	54.88
Spleen	53 "	159.9 "	33.14	201.8 "	26.25
Pancreas . . .	65 "	100.3 "	64.77	98.4 "	66.00
Right kidney .	101 "	142.0 "	71.11	321.7 "	67.13
Left kidney . .	115 "	150.1 "	76.61		
Adrenals . . .	25 "	23.5 "	106.29	17.3 "	144.21

TABLE II.

	L. C. R.	Five Equal-sized Cadavers (Boyd).	Percentage.
Body weight	61.4 kg.	60.4 kg.	
Height	172 cms.	174.8 cms.	
Brain weight	1,600 gms.	1,496.82 "	106.89
Right lung	385 "	1,230.34 "	31.29
Left lung	324 "	1,247.35 "	25.29
Heart	178 "	441.026 "	40.97
Stomach	112 "	230.852 "	48.51
Liver	914 "	3,196.419 "	28.59
Spleen	53 "	287.742 "	18.41
Pancreas	65 "	133.24 "	48.79
Right kidney	101 "	439.309 "	49.16
Left kidney	115 "		
Adrenals	25 "	31.183 "	80.16

Table II., which is also based on Boyd's statistics, was obtained by using Boyd's measurements of the viscera of three female and two male cadavers which correspond approximately to the height and weight of L. C. R. The average weights for the various organs obtained from the measurements of these five bodies are, however, probably a little too high in some instances, for the weights of some of the organs of one of the cadavers were undoubtedly considerably above the normal. Hence the truth in the case of L. C. R. probably lies between these percentages and those obtained by comparing Boyd's averages for paupers and lunatics in the decade between thirty and forty, with the measurements made on L. C. R.

Table III.

Percentages of the weight of the organs of the insane, paupers, and 5 comparable cadavers as given by Boyd, formed by the organs of L. C. R.

	Insane.	Paupers.	Five Equal-sized Cadavers.
Brain weight	129.95	117.09	106.89
Right lung	47.95	47.83	31.29
Left lung	47.58	46.90	25.97
Heart	61.20	53.60	40.36
Stomach	66.66	69.07	48.51
Liver	58.08	54.88	28.59
Spleen	33.14	26.25	18.41
Pancreas	64.77	66	48.79
Right kidney	71.11	67.13	49.16
Left kidney	76.61		
Adrenals	106.29	144.21	80.16

In Table III. it is seen that the percentages which the weights of the organs of L. C. R. formed of the average weights of the corresponding organs of paupers and of the insane, agree very well except in the case of the adrenals and the brain. However, as seen in Table II., the percentages derived from a comparison of the average weights of the organs from five approximately equal-sized individuals; chosen from both sexes and both classes of cases; with the weights of the organs of L. C. R. are considerably below the others. Reference to Table III. will make this evident. The discrepancy is especially marked in case of the lungs and the adrenals. In case of the former the percentages obtained by comparing the lung weights of L. C. R. with the average lung weight of the five selected individuals are undoubtedly much too low, but that obtained for the adrenals is undoubtedly much nearer the truth than those obtained by the other comparisons.

According to the old calculations of Liebig ('74) made on two men, the liver normally forms three per cent of the body weight, the spleen .35, the pancreas 1.5, the heart .7, the lungs 2.45, and the brain 2.35. The percentages of the body weight of L. C. R. formed by the corresponding organs are: 1.4; .08; .1; .28; 1.15, and 2.65 per cent. The very decided difference between the percentages obtained by Liebig and those found in L. C. R. make deductions quite impossible, except to emphasize the great need for a very large series of accurate measurements. The same remark holds for the adrenals, which according to Lucien and Parisot ('13), seldom weigh more than ten to twenty grams. Those of L. C. R., nevertheless, weighed twenty-five grams.

The microscopic examination necessarily was unsatisfactory because the tissue was not obtained immediately after death. Nevertheless, if for this reason pathologists were to exclude from their records all autopsies made no earlier than that on L. C. R., they would, to say the least, considerably reduce their material. This would be all the more the case if the degree of post-mortem decomposition rather than the time which elapsed since death were to serve as a criterion in this matter, as it really should. It is well known that the rate of post-mortem maceration and of putrefaction, are influenced very decidedly by temperature and ante-mortem conditions. In the case under consideration there was no infection and all other circumstances tended to delay the onset of post-mortem changes. The body had been deprived of nourishment for weeks, the extremities were cooled considerably before death, and if Retterer's observations on dogs can be applied, the general body temperature also must have been lowered several degrees. Besides, the dissipation of body heat was hastened by the cool atmosphere of winter. As already stated, aside from a little greenish discoloration on the lower abdomen which was undoubtedly largely due to the ante-mortem congestion of the subcutaneous tissues, there was absolutely no gross

indication of any putrefactive changes. This applied also to the small amount of gastric contents, which included some albumen.

Portions of almost all the viscera were excised and some of the organs preserved in toto in formaline. The smaller pieces were fixed in Zenker, Helly, and formaline, and the customary paraffine technic followed.

Before discussing the results a short description of the microscopic appearance of portions of various tissues and organs will be given.

Fat. — The dark red appearance of the subcutaneous and paravisceral fat was easily explained by the microscopic examination of the latter. As suggested by Figure 1, taken from a section of the perirenal fat, the hemorrhagic appearance of the subcutaneous tissues which contained nothing macroscopically recognizable as fat was undoubtedly not due to extravasations, but to dilatation of the capillaries which were engorged with blood. Not a single cell containing fat globules could be found in some sections and the adipose tissue had everywhere become so depleted that nothing remained but a fibrous web traversed by engorged capillaries. In case of the perirenal fat neither the cell boundaries nor the nuclei were fully preserved.

In the remaining remnants of the sub-pericardial fat the cell boundaries are better preserved and the fat cells are partly or wholly filled with a very finely-granular protoplasm. The appearance of this fat, in portions at least, recalls the appearance of the fat in the interscapular region of the common bat at the beginning of the winter season, except that the protoplasmic granules of the cells of the latter are larger, that some cells in the bat still contain fat and fat-droplets, that the nuclei are evident, and that the general appearance in the bat is that of a vital, well-preserved tissue. In some portions the remaining protoplasm of the fat cells of L. C. R. occupies the center of the cell and contains a vacuole or

fat-droplet. This fat is also invaded by acidophile cells with large vesicular nuclei. The remaining sub-pericardial fat also seems much better preserved than in some other regions and is not traversed by greatly dilated capillaries.

The appearance of the "fatty" marrow from the humerus was decidedly granular, as shown in Figure 2. Here and there a cell which still contains a small fat-droplet could, however, be seen and the appearance of the protoplasm resembled very closely indeed that from the interscapular region of the bat.

Muscle. — The extremely wide separation of the bundles is especially obvious in transverse sections. As shown in Figure 3, this is due to a reduction in size of the bundles which in many cases are too small for the fibrous sheaths. In other instances the bundles are completely gone and in still others, they are represented merely by a degenerated mass. These things give the sections a fenestrated and blurred appearance. In most fibers the striations are only faintly visible. In some a mass of degenerated nuclei is seen, while in other places a group of nuclei lies practically alone. Here and there one also sees an almost continuous succession of nuclei in the sarcolemma. A peculiar blurring of the protoplasm is also noticeable, and not infrequently the latter seems to be formed into granular masses. Many fibers, however, show the striations remarkably well.

The cardiac muscle was better preserved, but as shown in Figure 4, had undergone similar changes. Fragmentation was not present, but the myocardial fibers were separated more than normal thus producing a more open appearance.

Heitz ('12), also recorded a reduction in the size of the myocardial fibers in rabbits which had lost from twenty to thirty-six per cent in weight during starvation. Heitz, however, stated that the histological structure of the fiber had been modified only very slightly and only in small areas, and, strangely enough, concluded that inanition is felt as definitely by the myocardium as by the connective tissue.

Intestine. — The whole intestinal mucosa is so degenerated that one hesitates to say anything regarding it because of the lateness of the necropsy. Any one at all familiar with the changes usually present in the gastric and intestinal mucosa within eighteen hours post mortem can easily appreciate this. While the parietal cells of the gastric mucosa are better preserved the disintegration of the chief cells is marked. Some of the intestinal villi are filled with a mass of erythrocytes and these portions of the intestine show marked congestion. The mucosa is completely destroyed over large areas and the stroma of the villi is distended with blood, the erythrocytes of which are very well preserved, however.

The plexuses of the sub-mucosa show extreme congestion and this is also present in the solitary and aggregate lymph nodules which are greatly depleted.

The mucosa of the colon was completely devastated.

Liver. — Instead of being composed of continuous cords of closely-packed cells, disposed radially about the central vein, many hepatic lobules are composed of isolated cells and groups of cells with large intercellular gaps. As seen in Figure 5 numerous hepatic cells are too small for the reticular framework and the cords of cells are widely separated in many places. The disintegration of the cell cords is most evident near the central vein, the periphery of the lobule being always better preserved. Many of the hepatic cells are reduced to one-fourth or even to one-fifth their normal size. Others are represented by a small amount of protoplasm surrounding a nucleus, while still others are mere shadows of cells. A good deal of golden pigment is found intracellularly and in considerable masses also extracellularly.

Gilbert and Jomier ('06), found a rarefaction of the protoplasm of the "clear" and an increase in the number of the "dark" cells in the livers of dogs and rabbits which had been starved absolutely for periods of one to ten days. They noticed also an increase in the number of vacuoles and

the presence of perinuclear and peripheral clear zones. The nuclei were said to show no changes.

Iliac nodes. — The parenchyma especially of these lymph nodes is very much depleted and some portions of the nodes are comprised of the collapsed coarser framework merely. Germinal centers are absent and large trabeculæ and large sinuses are especially evident. Some of the latter contain granular detritus. Few degenerated cells are found, however, although some yellow pigment is present. Only a few polymorphonuclear leucocytes are seen, but a good many acidophile cells with finely granular protoplasm and round vesicular nuclei are present.

The parenchyma is so depleted that one can actually count the cells in sections ten microns thick in most portions of the sections. The nuclei of the lymphocytes contain few chromatin granules, stain lightly, and look more transparent than usual, some of them appearing as empty vesicles.

Prevertebral nodes. — The prevertebral nodes seem somewhat better preserved, but show some polychromatophilia. They, too, contain no germinal centers, phagocytes, or giant cells. Large cells with a large, oval, vesicular nucleus, which look as though they might have an endothelial origin, are usually numerous. These cells are contained in both sinuses and parenchyma. These portions contain large acidophile cells and masses of degenerated erythrocytes.

Bronchial nodes. — The bronchial nodes are not so depleted as might be expected, but show considerable pneumonokoniosis. They contain almost nothing but small lymphocytes. Some portions of the abdominal lymph nodes are wholly depleted, being represented merely by a folded mass of trabeculæ and connective tissue.

Spleen. — Malpighian corpuscles are practically absent, for only a few can barely be distinguished. Jolly and Levin ('12) found that acute starvation in cats and guinea-pigs results in the thinning of the pulp cords and a reduction in the lymphocytes. The latter also became clearer, and the venous sinuses

were often filled with large phagocytic cells, which were stuffed with erythrocytes. In birds the capillary capsules disappeared.

Many erythrocytes are distributed about the parenchyma, but the splenic sinuses are not evident. The erythrocytes, though various-sized, are quite well preserved. The vessels are empty, and only a few giant cells and phagocytes are found, though comparatively large amounts of pigment are present. The staining reactions seem normal except that the stain is a little fainter than could be expected. Nucleated reds were not found.

Bone marrow. — As judged by specimens taken from a rib and humerus the depletion of the bone marrow was extreme. Large areas were wholly devoid of cells and looked essentially as shown in Figure 6. Normal fat cells were present nowhere in the specimen examined and in some portions only a web of fine reticulum remained. This agrees very well with the conclusion of Sedlmair ('98) that the fat of the bones is lost in starvation, just as is the other body fat. Here and there a small field of marrow cells of the character of lymphocytes, which were mixed with a larger number of erythrocytes, was found. Although the erythrocytes predominated no nucleated reds were present. Many capillaries were engorged with blood.

According to Roger and Josué (1900), Bizzozzero, Torre, Goyer, Neumann, and Stolz, all have described a lymphoid state of the marrow, a mucoid degeneration of the cells and walls of the vessels, and the disappearance of the fat in starvation. Roger and Josué, who subjected some rabbits to absolute starvation for a period of five to seven days, found that the medullary cells had proliferated abundantly, giant cells were numerous, the fibrils large and edematous, and the fat in the fat cells had been replaced by a granulur substance which stained pink with eosin. In some cells a drop of fat was still visible, but the nuclei of all fat cells were decidedly degenerated, some being mere shadows.

Kidney. — Upon inspection of a stained mounted section with the unaided eye, the renal parenchyma is seen to be studded with little red dots. Upon microscopic examination these are found to represent hemorrhages, but the blood corpuscles are not found in the intertubular areas, but lie in the connective tissue framework which was previously occupied by the tubule itself. Such a condition could, of course, not result from post-mortem maceration. It is, to be sure, a matter of common knowledge that the renal parenchyma undergoes degeneration very soon after death and the study of Schmitter ('05) shows how very early maceration changes can be detected. Nevertheless, one cannot believe that erythrocytes could take the place of the parenchyma subsequent to the post-mortem autolysis of the latter.

It is true that an astonishing amount of autolysis has taken place in the kidney, but this autolysis is not the only thing noticeable. In addition to the hemorrhage a very pronounced atrophy and degeneration of the renal parenchyma has occurred. Some of the glomeruli are completely destroyed, only blood corpuscles being left, while others are very much shrunken. Some portions of the kidney are hemorrhagic.

Adrenals. — In some portions the glomerular cells of the adrenal are mere webs and great cell disintegration is present. The atrophy is very marked and many shadowy cells are seen. The parenchyma contains many open spaces, the medulla is not only greatly reduced in size, but is greatly vacuolated, and almost completely destroyed in places. When the cytological preservation of these adrenals treated by the best methods and taken from a practically fresh body eighteen hours after death, is compared with similar material from guinea-pigs and especially with that of the aberrant adrenal taken nine months post mortem from a dissecting room cadaver which had not been embalmed till three days after death, the contrast simply is amazing. (Such a specimen was described by Meyer, Anatomical Record, '17.)

Hypophysis. — The anterior lobe of the hypophysis shows great congestion and contains hemorrhagic areas similar to those found in the kidney. The epithelium shows great reduction and degeneration similar to that shown in the adrenals, and in some places is displaced completely by blood. Only a few small masses of colloid were found. A portion of the congested area is shown in Figure 7. A low degree of chromatophilia is still evident.

Thyroid. — The thyroid is in very good histological preservation, but the epithelium of the acini is extremely low. Indeed, in some acini it is so low that one has to look very carefully in order to recognize it. There is practically no desquamation of epithelium and no pyknosis. Rarely groups of four, six, or twelve small acini containing but little colloid are found, but the rest of the thyroid is really converted into a mass of colloid which greatly distends the acini and suggests exhaustion. The picture is that of atrophy with no signs of proliferation anywhere.

Parathyroids. — The parenchyma of the parathyroids looks disordered and as though it had been depleted, but otherwise it is well preserved. Some portions contain a good deal of colloid. This is represented in black in Figure 8. Other portions contain faintly-staining acidophile cells, which are very evidently in differing stages of degeneration.

Pancreas. — The parenchyma, except the pancreatic islands, is quite well preserved. The islands are small and degenerate, and are recognized with difficulty only. As shown in Figure 9 *a*, a fused mass of degenerated cells is all that is left of some islands. Others are somewhat better preserved (see 9 *b*), but all are small and have shrunken away from the surrounding connective tissue.

The histological condition of the pancreatic islands suggests that glycosuria should have been present, but this was not the case, for as Professor Swain suggested, the need of the organism for glycogen was so great and the stored glycogen so long exhausted, that a diabetic condition did not arise.

Lungs. — Aside from some edema and congestion the lungs show nothing of special interest. The edema largely accounts for the heavy weight.

Salivary glands. — Many of the peripheral acini look shrunken and the connective tissue framework surrounding them is too large. Some of the degenerate-looking acini take an acidophile stain. Other acini have almost completely disappeared, although these changes apparently have affected only relatively small portions of the gland. The remaining protoplasm is often toothed or cogged as in macerated specimens, and the nuclei are collected near the center of the cell. Vacuoles are quite common and large. The mucous cells seem better preserved. Accumulations of lymphocytes are found about some of the ducts. Jolly and Levin ('11) observed accumulations of lymphocytes around degenerating corpuscles of Hassal in the thymus of birds subjected to acute starvation also. Some of these accumulations were spoken of as enormous, and they state that a complete lymphocytic transformation of the thymus can be affected by eight to nine days of acute starvation.

The submaxillary and sublingual glands show somewhat less marked though similar changes.

Cartilage. — The nuclei of the cartilage cells of the epiglottis are shrunken to very small size, or are wholly absent even. Often the spaces formerly occupied by the cartilage cells are empty, while in other cases the nuclei are represented by very irregular small masses. The greatest degeneration is evident in the interior of the cartilage. The cells on the border are much better preserved, although most of the nuclei are extremely small and shrivelled. Exactly the same phenomena are evident in the cartilage of the trachea and that these appearances are not due to post-mortem changes is evidenced by the appearance of the nuclei in the surrounding tissue, for even the cilia of the columnar epithelium of the trachea are well preserved.

Brain. — Although the brain weighed 1,600 grams it is evident from the appearance of a portion of the cerebral cortex taken from the frontal lobe and represented in Figure 10 that this large weight was not by any means wholly due to the weight of the histological elements, but to the presence of a large quantity of fluid in the substance of the brain. In many places of both the brain and cord there are spaces of considerable size, many of which are perivascular, which at first give one the impression of being due to shrinkage in fixation. But the atrophic cells, the reduction in amount or even the complete disappearance of the protoplasm, and of entire cells, the fresh condition of the brain at autopsy as well as the care taken in its preservation make such an origin wholly improbable. Moreover, as shown in Figure 11, the change in the motor cells in the anterior horn of the cord is entirely comparable, thus increasing the likelihood of these changes being largely the direct consequence of the inanition itself. The nuclei are small and fragmentation of some nuclei apparently has taken place in these cells, and considerable loss of material has occurred. Some of the cells are mere remnants and all are surrounded by very wide clear zones. Clear areas are also scattered throughout the gray substance and many nuclei are barely visible.

Because of the long time since death (eighteen hours) no examination of the neurofibrillar network of these cells was made. Riva ('06), however, found it particularly resistant to starvation changes as evidences in the anterior and posterior horn cells of rabbits and dogs starved up to forty-eight days. Riva found that the network does not become disorderly, but vacuoles form in it causing the fibers to disappear in these places. Donaggio ('06), on the other hand, found that inanition in rabbits accompanied by exposure to severe cold caused great disorderliness in the neurofibrillar network of anterior horn cells.

Barrows ('98), who does not state the duration of abstinence, also found a decided shrinkage in the size of the cells and nuclei, especially in the nucleoli in famished rats. The

shrinkage amounted to about twenty per cent. Vacuolation was present and the nuclei and nucleoli were absent in some cells.

Even in rabbits and guinea-pigs which succumbed in twelve to eighteen days, respectively, Marchand and Vurpas ('01) found the anterior horn cells shrivelled and vacuolated in places. The protoplasm and the degenerating nuclei stained deeply, the cell processes were shorter and less numerous, and the nuclei sometimes were wholly absent. The lesion above all was found to be an atrophy of the cell and the cell processes. No signs of inflammation were present, but the vessels were dilated and engorged with blood.

Schaffer ('97) also found chromatolysis, vacuolation, and pyknosis. Vacuolation was present especially in the spinal cord of these rabbits and always occurred in the periphery of the cell body, thus giving the nerve cells a toothed or cogged appearance. Similar effects were observed by Coen and Monti and also by myself in some of the glands.

As shown in Figures 12 and 13, taken from the fiber tracts of the cervical cord and a root of a cervical nerve, respectively, these structures contain many more neuroglial cells than are normally present. Rarely, a group of these cells is seen surrounding a capillary and one can scarcely escape the conclusion that they are neuroglial cells which have migrated there or endothelial cells which have arisen there. Although moderately dilated capillaries and some slightly hemorrhagic areas were seen, no evidences whatever of phagocytic activity were present.

Considerable shrinkage and vacuolation of the myelin are present, and the cross-sections of the fibers are irregular in outline. In some fibers the myelin seems to be almost wholly gone. Peri ('92), who starved rabbits, cats, and dogs for intervals varying from two to thirty-four days with a subsequent loss in weight of a few to approximately forty-five per cent, also noticed a definite atrophy and reduction of myelin in the peripheral nervous system in animals which had been starved a long time.

Since material was not obtained until eighteen hours after

death it is, of course, impossible to determine exactly which of the changes above recorded were present at the time of exitus. Some of the changes found were undoubtedly partly due to post-mortem autolysis. In order to determine the changes which occur under approximately similar conditions, however, a comparison was made between the tissue obtained from L. C. R. and tissues taken from dissecting room cadavers and also from guinea-pigs, five to twenty-five hours after death. As already stated the intracranial adrenal taken from a dissecting-room cadaver nine months after it was preserved in the ordinary routine way, showed no comparable changes, in spite of the fact that this body had not been preserved until three days after death. Lymph nodes and some other tissues removed from dissecting-room cadavers many months after death also were far better preserved than the nodes from L. C. R. Nor were corresponding changes found in a guinea-pig twenty-four hours after death, although the animal was killed with a full stomach and intestine, thus assuring bacterial activity and a good opportunity for the onset of putrefactive changes.

Through the coöperation of my colleague, Professor Ophüls, I was also enabled to examine approximately one hundred slides of material taken from autopsies fifteen to twenty-six hours post mortem. Although not every organ was represented in this material it was sufficiently representative to justify the conclusion that those changes present in the organs of L. C. R., which might be attributed to maceration, could by no chance be due to the ordinary post-mortem maceration.

The conclusion that ordinary post-mortem changes do not and, in a large measure, cannot account for the conditions found in L. C. R., also receives some support from the fact that Statkewitsch ('93), who autopsied most of his large series of experimental animals immediately after death, nevertheless concluded from a comparative examination of tissue from a dog which was still warm twenty-four hours after death, that post-mortem changes could be excluded even under those conditions. Had Statkewitsch done a less

comprehensive and careful investigation one would be justified in calling this conclusion incorrect, for marked post-mortem changes do, of course, occur within twenty-four hours after death, especially if the body is kept warm. Nevertheless, I am certain that much material used by pathologists is not in as fresh a condition as was that taken from L. C. R., even in cases in which the necropsy is done in less than eighteen hours after death.

Hence, although I am fully aware of the fact that many other matters must be considered in making comparisons, I firmly believe that only two explanations can account for the marked changes found in this cadaver. The perfectly fresh odor of the body, the entire absence of abdominal distension, the low temperature, the depleted condition of the body, and the ante-mortem cooling, all minimized the effect of the ordinary putrefactive changes. Hence, truly remarkable cell changes must have been present before death, and, perhaps, began long before death, or, on the other hand, post-mortem autolysis must have been hastened tremendously by the starvation. The latter supposition receives some support from the conclusions of Cesa-Bianchi ('09), that degenerative changes are hastened by the accumulation within the cells of steatogenous substances. Nevertheless, the many experimental studies made on the effects of prolonged starvation, show that such a long period of complete abstinence as sixty-three days, undoubtedly produces marked ante-mortem changes in itself.

It is not my purpose to discuss the whole literature on starvation, not even on its morphological side, partly because a good deal of it is in Russian, but also because the state of exhaustion represented in L. C. R., no doubt far exceeds what can ordinarily be obtained and what was obtained experimentally. Moreover, in the case here recorded we are dealing with an end result only, and the finer cytological changes can hardly be discussed, because of the delay in obtaining the autopsy. Animals, other than those which hibernate, will usually not live for so long a period as sixty-three days. It is true that Howe and Hawk ('11),

were able to keep a dog alive during a fast of one hundred and seventeen days followed by recovery of the original weight and a subsequent fast of one hundred and four days. The survival of the animal for so long a period of time was probably due, however, to the fact that water was supplied by gavage. Most of the laboratory animals used in the older experiments lived or were allowed to live a comparatively short period, ranging from one to five or six weeks.

It is true that in the case of Necturus maculatus, Rogers and Smallwood ('11) found practically no cytological changes after four months of complete starvation. But after six months marked changes were seen in the cord, stomach, and the intestine. The gray matter of the cord disappeared, the nerve cells shrank and became vacuolated. From a study of acute inanition in young and mature rabbits during a period of five to ten days, Morpurgo ('89), concluded that mitoses stopped earlier in the liver, pancreas, gastric glands, and kidneys than in other organs. Cesa-Bianchi ('09), found that a prolonged period of sub-maintenance feeding in white mice which produced severe cachexia in ten to twenty days, resulted in a loss of weight of not over forty per cent. Those that lost only thirty per cent invariably recovered. Cesa-Bianchi found that such protracted sub-maintenance feedings with a gradually increasing reduction produced all the changes which can be produced by "osmotic disturbances and aseptic autolysis." He recognized two changes only: (1), a transformation of the cytoplasm into drops and granules such as can be produced by osmosis; and (2), severe changes in the cytoplasm and nucleus which result in a myelinization. Cesa-Bianchi thinks that a cell which has undergone only the former change can recover, but that myelinization invariably leads to cell death. He also concluded that the intensity of the changes is in direct proportion to the duration of the starvation and may in advanced cases lead to complete destruction of the cell. He found nucleopyknosis and karyorrhexis common in the kidney but not in the liver.

The extensive experiments made by Statkewitsch ('93) on

cats, dogs, rabbits, pigeons, frogs, lizards, and a turtle contain much that bears on the present case. The loss of weight in the forty-four animals used by Statkewitsch varied from five to fifty-one per cent.

Statkewitsch found atrophy and degenerative processes present in muscle and glandular elements, and concluded that the glands suffered more than the muscles. The latter showed the cloudy swelling and granular degeneration. The liver showed the greatest changes, then came the kidney, the parotid, and submaxillary, and then the pancreas. Although fatty degeneration was not present in the muscle, it was found in the liver and kidney. Besides these things, a degeneration into pigment and cellular disintegration, were also present. But the most marked changes were found in the cortex of the kidney, where besides these changes almost all elements were changed into "helle Zellen."

Next to the great depletion and destruction, the number of such transparent cells in the specimens from L. C. R. is perhaps the most striking thing. The pancreas contained heaps of these cells which Statkewitsch says were called pseudo-follicles by Podwyssowsky, and which, according to him, have been taken for lymphatic structures. The only thing comparable found in L. C. R. were the degenerated pancreatic islands, which might remotely simulate lymphatic islands if only the nuclei remained. Statkewitsch found no mitoses in starving cells, which is in entire agreement with what was observed in L. C. R.

It was a surprise to me that aside from the large amount of colloid, and the fact that the acini were not small, the thyroid gland did not show the changes found in the careful and comprehensive study of the rat by Jackson ('16). Nor did the thyroid show the autolysis spoken of by Marine ('15) as occurring in a few hours after removal of the gland from the body. This may, to be sure, be due to the fact that the thyroid was not removed until fixed, eighteen hours post mortem, and also to the rapid cooling of the body. Nor did the colloid show the fatty degeneration reported by Missroli ('11) in rabbits after prolonged fasting.

A surprising correlation between the microscopic picture presented by the various organs and the percentage loss in weight of these organs becomes evident on comparing the two. The body fat was practically depleted, as judged by gross appearances, and as shown in Figure 1 the histological picture strikingly confirms this. An inspection of Figure 3 also bears out the high percentage loss in the body musculature, as implied by the great loss of body weight and by the losses in the musculature, ascertained by Chossat ('38), Sedlmair ('98) as cited by Voit ('04), in the case of pigeons, cats, and dogs, respectively. From Table IV. it will be seen that the voluntary musculature generally loses more than the cardiac, and that the liver, kidney, spleen, and pancreas form an increasing series. This is quite in harmony with the microscopic pictures presented by these organs.

TABLE IV.

Percentage loss of fresh organs.[1]

	Chossat.	Sedlmair.	Voit, C.	Voit, E.[2]	L. C. R.
Skeleton..........	3	10.4	14	5	......
Muscle...........	42	57.9	31	42	40.7 (Whole body)
Brain and cord....	1	2.2	3	22	6.89 (Brain)
Heart............	45	34.0	3	16	40.36
Spleen...........	71	68.5	......	57	18.41
Liver............	53	59.8	54	50	28.59
Pancreas.........	64	55.1	......	62	48.79
Kidney...........	32	50.9	......	55	49.16
Lungs............	32	20.5	......	29	28.63
Fat..............	......	......	97	......	......

1. The marked discrepancies in the percentages are largely due no doubt to the decided differences in the duration of the periods of starvation and in a minor degree to the conditions of starvation and to the age and species of the animal. The calculations of Chossat were based on pigeons, of Sedlmair and Voit, C., on cats, and of Voit, E., on dogs.

2. Loss of fresh fat free organs.

Voit, C., suggested that the differences in loss of weight of different organs can be explained by assuming that certain organs live at the expense of others, as is apparently the case in the spawning salmon. The skeleton always loses little in weight during starvation. Some of its substance is replaced by water, however. A very peculiar observation is that made by Chossat on pigeons and confirmed by Sedlmair on cats, that the spinal cord loses much more in weight than the brain. The loss of weight of the different organs and fat taken in the fresh state apparently varies from one to ninety-seven per cent of the total. Hence it is evident that the true loss in weight of an organ cannot be determined without taking into account the amount of fat present at the beginning. Moreover, the increase in the relative amount of water during starvation must also be taken into consideration because it masks the actual loss in the formed elements.

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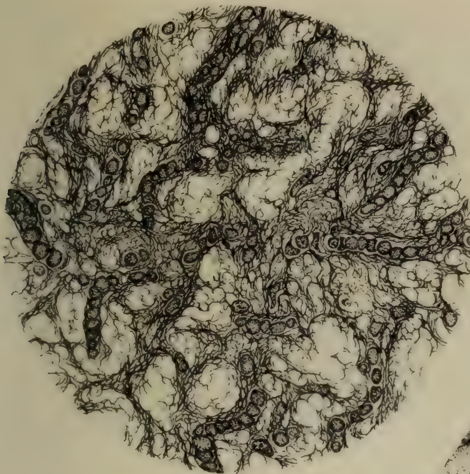
DESCRIPTION OF PLATES III.—V.

PLATE III.:
1. Perirenal fat. x 720.
2. Marrow from right humerus. x 475.
3. Pectoralis major muscle. x 630.
4. Cardiac muscle. x 720.
5. Liver. x 720.

PLATE IV.:
6. Marrow from a rib. x 375.
7. Anterior lobe of hypophysis. x 750.
8. Parathyroids. x 630.

PLATE V.:
9. Pancreatic islands. x 630.
10. Cerebral cortex. x 375.
11. Anterior horn cells. x 475.
12. Fiber tracts of the cervical cord. x 475.
13. From a cross-section of a dorsal root of a cervical nerve. x 920.



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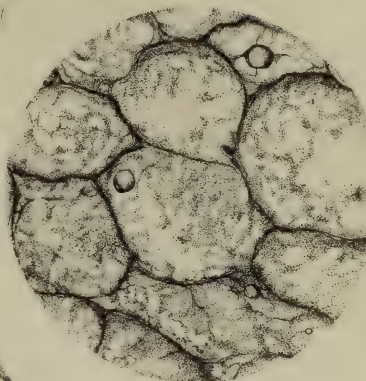

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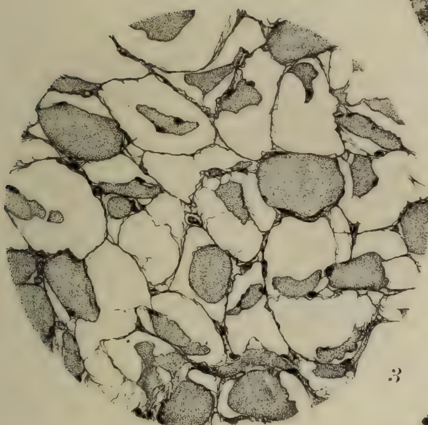

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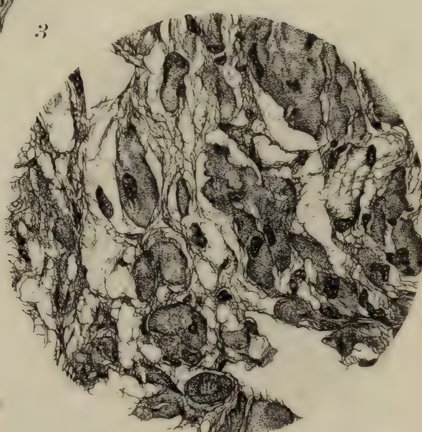

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Meyers. Prolonged inanition.

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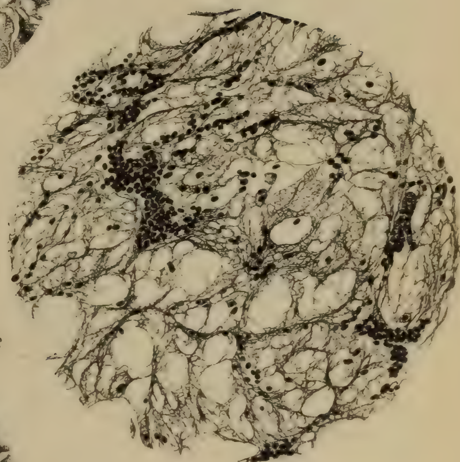

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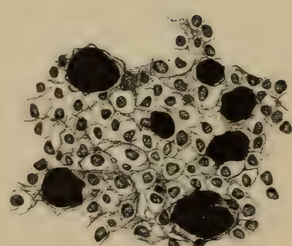

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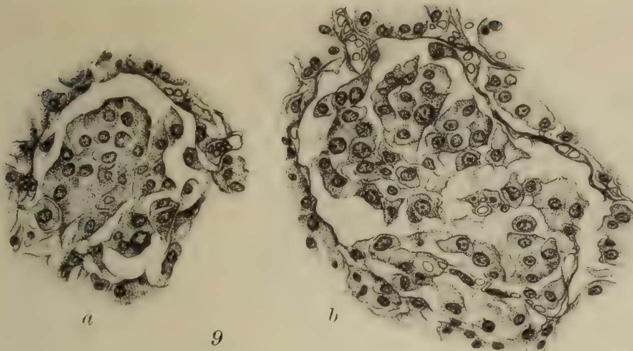
Meyers.

Prolonged inanition.



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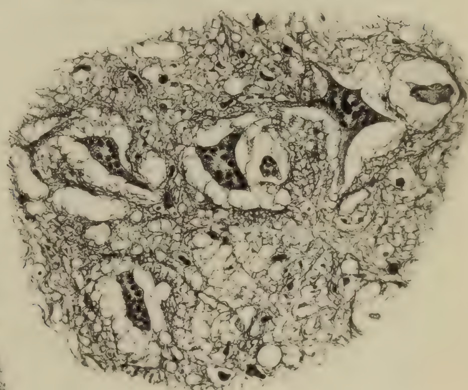

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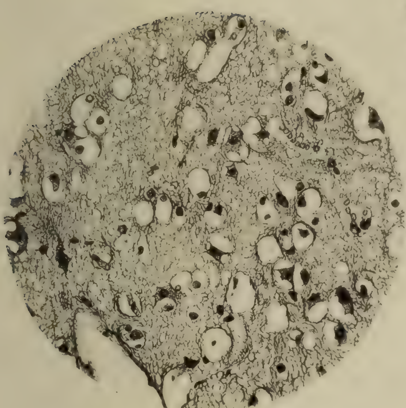

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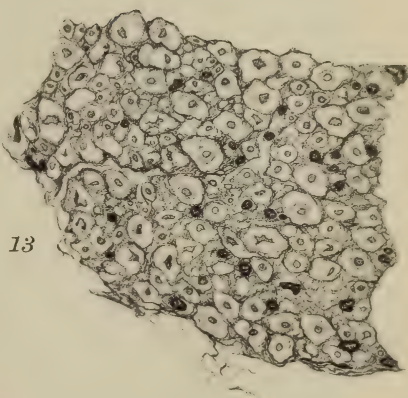

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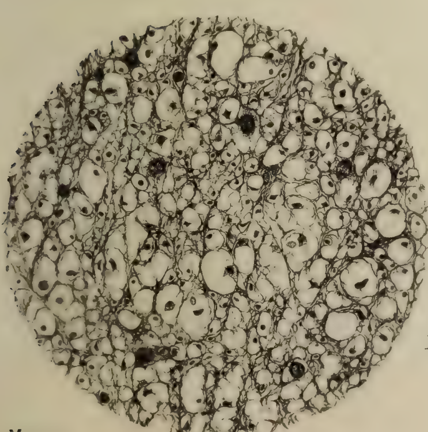

12

THE PART PLAYED BY THE GOBLET CELLS IN PROTOZOAN INFECTIONS OF THE INTESTINAL TRACT.*

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That amebæ may frequently pass from the contents of the intestines through the epithelial wall, and produce extensive lesions in the sub-epithelial regions, notably in the sub-mucosa, has long been recognized. More recently the present writer[1] has been able to show that a type of infection, agreeing in many respects with amebic infection, may be caused by one of the flagellated protozoa, *Trichomonas*. In this case the organisms, which are almost invariably present in the intestinal tract of animals and birds, in some way penetrate the epithelial wall and undergo a most prodigious development in the deeper mucosa, sub-mucosa, and even the muscular tissues of the intestinal wall; they may at times penetrate even to the serosa. In the region of the mucosa they may frequently develop to a considerable extent without affecting the major portion of epithelium, but in severe cases this is usually desquamated to a considerable degree. In this region the cores of the villi become crowded with the parasites, multiplying by a process of autogamous reproduction. The muscularis mucosæ is readily passed by the organisms which throng the sub-mucosa, occupying every available space in the reticular tissue. In the muscle tissues they penetrate between the fasciculæ, and even between the fibers, spreading them apart and causing a marked increase in the size of the regions of both circular and longitudinal muscle areas. Such infections as this, which may occur in many species of domesticated birds and in some wild species, are typified by many cases of the so-called blackhead of turkeys, a common disease which causes great losses each year in the turkey-raising industry of this and other countries.

In infections of this sort, as well as in amebic infections of

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the intestinal tract of man, one of the interesting questions concerns the means by which the parasites succeed in penetrating the epithelial wall in order to despoil the sub-epithelial tissues. Two possibilities have suggested themselves: (1) Are these organisms able by their own movements to effect an entrance between epithelial cells? (2) Is the way prepared for their invasive activity by other intestinal residents such as the coccidia (epithelium-despoiling sporozoa) among the protozoa, or such as some of the intestinal worms?

There is no evidence at hand to recommend either one of these hypotheses. From what is known of the structure, habits, and mode of life of the amebæ and flagellates of the Trichomonas group, it would appear a mechanical impossibility for them to penetrate an intact epithelium. In the second place, while some sort of a preparatory action (in destroying portions of epithelium) on the part of coccidia or parasitic worms cannot be denied, there is no evidence that such damage, where it occurs, favors the invasion on the part of the flagellates.

Within the past year the writer's studies upon protozoan infections in birds have led him to regard with special interest the epithelium of the wall of the ceca, with the hope that such a detailed study might lead to the discovery of the actual means whereby the flagellate, Trichomonas, forced a passage through the epithelial bank and set out upon its tissue-destroying career in the sub-epithelial regions. This study has proved unexpectedly successful, and it is the aim of this paper briefly to point out the actual avenue of entrance of the parasites through the goblet or chalice cells (gland cells) located in the epithelial walls deep in the crypts of Lieberkühn.

It will be recalled that the epithelium of the ceca of birds is thrown into deep folds or crypts, as is usual in the intestines of animals. The blind end or fundus of these crypts approximates closely upon a narrow region of muscle tissue, the muscularis mucosæ, surrounding the cecal cavity. Outside of this lie (in order) the sub-mucosa, the region of

circular muscle fibers, the region of longitudinal muscle fibers, and lastly the serosa. Among the columnar epithelial cells, which make up the major portion of the epithelial wall, may be observed (Fig. 3) unstained areas or vacuoles which represent the gland or goblet cells whose function is to pour their secretions into the crypts.

Under normal conditions the approximating banks of epithelium lie close together as indicated in Figure 2. Just before the period of actual tissue-invasion, however, a change is noted, as shown in Figure 3. The epithelial walls of the fundus of the crypt are made to bulge by reason of the tremendous number of flagellate trophozoites which crowd the lumen. These trophozoites have been formed in multitudes of mother cells (Fig. 4) present in the fluid cecal content (a condition invariably preceding actual infection), and have migrated up the tubules gathering in vast numbers at the blind ends. They possess the usual Trichomonas features — three anterior flagella and one posterior, a vibratory membrane, nucleus, blepharoplast, rhizoplast, chromatic line, chromatic blocks, axostyle, and nucleus. They are actively motile and measure from eight to ten microns in length. The shape is at first crescentic (Fig. 1), later oval. These are the organisms that accomplish the penetration of the epithelial wall.

When one examines with some care the epithelium bordering the gorged crypts it will be seen that, although the majority of the goblet cells are without visible contents, a few in many of the crypts contain from one to three flagellates, possibly more (Figs. 6, 10). In these cases there is usually a broad opening from the goblet cell into the lumen of the crypt (Fig. 5). In many cases, moreover, there is an opening, not only into the crypt, but also through the basement end of the goblet cell into a space (partly an artefact due to shrinkage) present between the epithelium and its basement membrane. Furthermore, within this space may be seen, in the case of different crypts, varying numbers of flagellate trophozoites (Fig. 8) that have, to all appearances, recently passed through the goblet cells.

If one examines a sufficient number of crypts parasites may be found in all stages of passage, some entering the cell, others within it, and still others emerging on the further side of the epithelial wall. These pictures are so common at this initial stage of the infective process, and so clear in their detail that there can be no hesitancy in concluding that it is by the avenue of the goblet cells exclusively that the flagellates gain lodgment in the sub-epithelial tissues where their maximum development takes place.

In connection with this phenomenon it is of interest to speculate as to the mechanism whereby the trophozoites carry out this cell-invasion. In the first place it is to be observed that this invasion occurs more commonly in the case of those crypts whose walls are distended with parasites. It is clear that a considerable internal pressure must be present in these cases, and one might assume that this pressure was sufficient to force the parasites bordering the epithelium into any receptive pocket, such as a goblet cell. This could easily occur, and doubtless does occur in many instances. Under these conditions the flagellate trophozoite is playing the part of a passive cell parasite. Moreover, even if this were true, it does not seem probable that the same pressure that forced the flagellate into the mouth of the goblet cell could also be instrumental in forcing the same parasite out of the basement end of the cell and into the sub-epithelial region, since in the cell itself the parasite has a considerable space available for free movement.

This circumstance would seem to suggest that the flagellate trophozoite actually possesses inherent invasive powers, and further evidence in favor of this view is derived from observations on the goblet cells of those crypts, which, though containing a moderate number of flagellates are not packed with them. In these cases also, in which the factor of pressure is presumably absent, the invasion of the goblet cells commonly occurs. This evidence seems sufficient to warrant the conclusion that pressure within the crypt is of minor significance in furthering the invasion of the goblet

cells, but that the cells are entered as a result of the natural invasive ability on the part of the flagellate trophozoites. At the same time it is apparent that, for some reason at present unknown, the entering of the gland cells takes place only, or at least chiefly, in the region of the fundus. Although the organisms may be present over a considrable extent of the crypt extending toward the cecal lumen, invasion at these points seems seldom to occur.

Although the presentation of the above data regarding the part of the goblet cells concludes the aim of the present note, it may be pertinent to add a word regarding the effect of this invasion by flagellates upon the epithelium itself as the infection proceeds.

The first of the flagellate invaders to penetrate the epithelium of the fundus may be seen, as pictured in Figure 8, lying between the epithelium of the crypt and the basement membrane. When once an avenue has been made through a goblet cell, it is clear that many parasites follow the same path and quickly become distributed (in three planes) on the back side of the wall. It seems that the epithelial cells must become detached from the basement membrane very easily, since, with the continued passage of the parasites, they begin to accumulate between the membrane and the wall. As time passes the number becomes sufficient to loosen the epithelium at all points surrounding the crypt, and at this stage one of two things may occur. In the first place the effect of the presence of many parasites behind the epithelium may be to initiate a disorganization of the epithelial cells (Fig. 9), now bound merely by their intercellular substance. As a result even this means of adhesion is weakened and other parasites commence to penetrate the wall by intercellular routes. Finally, the greater number of the flagellates which were formerly on the inner side of the epithelial wall come to be on the outside, and the wall itself begins to break down, the cells degenerating with the liberation of their nuclei (Fig. 11).

In the second place, the wall may not become disorganized,

but, with the direction of pressure brought to bear by the parasites reversed, the epithelium of the fundus is gradually forced out of the crypt space, toward the central lumen of the cecum. Here it may become imprisoned by subsequent consolidations and finally degenerate if the infection is sufficiently prolonged.

But, by the time the changes alluded to above are taking place, the parasites are also extending their course into the deeper tissues. First the basement membrane is penetrated, but seldom until the body of parasites lying in the crypt space has become considerable. The balance of the mucosa is quickly overrun and soon the muscularis mucosæ is reached. This appears to impede the course of invasion only temporarily, for soon the parasites have penetrated it and are developing in the sub-mucosa. At the same time they are spreading down through the reticular tissue of the cores of the villi, and, having reached the tips, break in behind the epithelium which becomes separated from the basement membrane at these points and falls into the cecal canal.

At the site of the old crypt the area becomes consolidated with the presence of the parasites, phagocytic cells, and proliferating round cells. Finally, no trace of epithelium can be observed in all this area where were formerly the blind ends of the crypts. In extreme cases the entire mucosa peels off at the level of the muscularis mucosæ, and the latter comes to form the wall of the cecal canal.

Conclusion. — This much then can be said regarding the avenue of infection in the case of Trichomonas: it is through the goblet cells of the fundus of the crypts of Lieberkühn. Intercellular invasion takes place only after the first ranks of the parasites have passed through the wall, and the disorganization of the epithelium has commenced. To what extent the same avenue may serve for amebæ cannot be stated at this time. Amebæ have been seen by the writer deep in the crypts where they may be present in small numbers with the flagellates, some of which they ingest.

They have not been seen by the writer either in the goblet cells or in the deeper tissues, and are probably of slight significance in the pathology of intestinal infections in poultry. *Ameba meleagridis* of Theobald Smith is not an exception to this statement, since this organism is not really an ameba, but merely the late trophozoite stage of the parasitic flagellate forming the subject of this paper.

Notwithstanding the fact, however, that amebæ have not been observed to penetrate the goblet cells, the data presented above surely suggests the possibility; and this, as in the case of Trichomonas, may be eventually recognized as the avenue of infection in intestinal amebiasis. It should be pointed out, however, that the sites of such invasion through the goblet cells are frequently quite localized, and marked activity of the parasites in the deeper tissues may be observed in regions far removed from their original point of entry. The extensive Trichomonas lesion is the end-result of the coalescence of a variable number of necrotic areas, each of which has had its own path of infection in an area frequently much limited in extent.

SUMMARY.

The present paper presents observations which demonstrate that, in the case of the flagellate protozoan, Trichomonas, the cause of an acute malady of several species of birds, notably the turkey (blackhead), the avenue of invasion of the sub-epithelial tissues is the goblet or chalice (gland) cells located in the fundus of the crypts of Lieberkühn. It is suggested that the passage of the parasites through these cells is not inadvertent, but due to a natural invasive power present in the motile flagellate trophozoite which accomplishes the infection of the deeper tissues.

REFERENCE.

1. Rhode Island Agr. Expt. Sta. Bul. 166, 1916.

DESCRIPTION OF PLATE VI.

FIG. 1. — Showing the young flagellate trophozoites, manifesting the crescentic form lying in the cecal content previous to the invasion of the tubules.

FIG. 2. — Longitudinal section through a normal crypt showing close approximation of the epithelial walls.

FIG. 3. — Longitudinal section through the fundus of a crypt distended with flagellates.

FIG. 4. — Showing the spore mother cells, containing daughter nuclei as they occur in the intestinal content previous to the liberation of the trophozoites (Fig. 1).

FIG. 5. — Showing a trophozoite in the act of entering a goblet cell at the fundus of a crypt. The nucleus, blepharoplast and chromatic line are observable.

FIG. 6. — Oblique section through the fundus of a crypt showing a goblet cell containing a flagellate. Other parasites occupy the lumen.

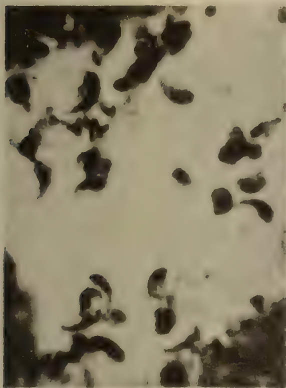
FIG. 7. — Showing a goblet cell invaded by flagellates which have pressed the nucleus out of the basement end. The flagellates do not appear in the photomicrograph because they were in a different plane (focus) from that of the nucleus.

FIG. 8. — Longitudinal section through the fundus of a crypt showing flagellates packed in the lumen; also, after having penetrated the wall, lying at points indicated (X) between the wall and the basement membrane.

FIG. 9. — Oblique section through the fundus of a crypt in which many flagellates have penetrated the epithelial wall and now lie behind it; the epithelial cells are beginning to degenerate.

FIG. 10. — Longitudinal section through a portion of the fundus of a crypt showing a goblet cell which has become an avenue of invasion into the deeper tissues. The epithelium has shrunk away from the basement membrane and in the cell are observed three flagellates.

FIG. 11. — Site of a former fundus of a crypt at the beginning of consolidation of the tissue.



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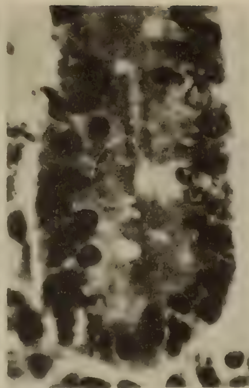

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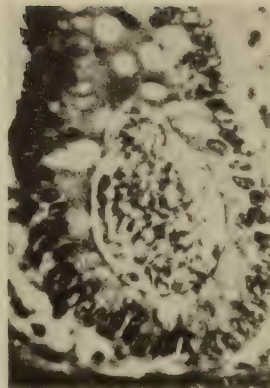

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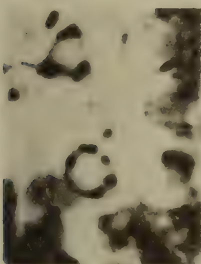

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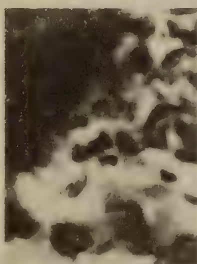

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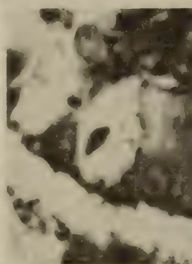

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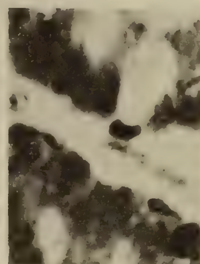

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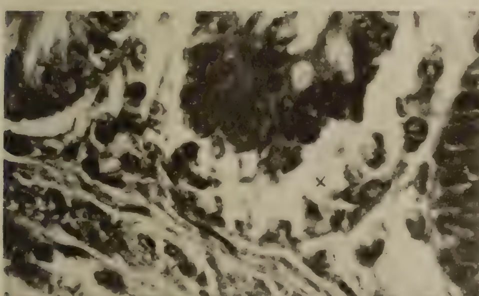

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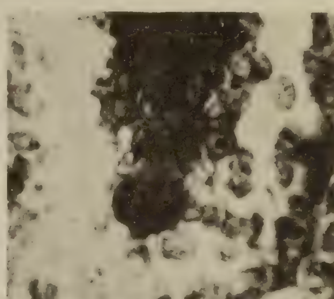

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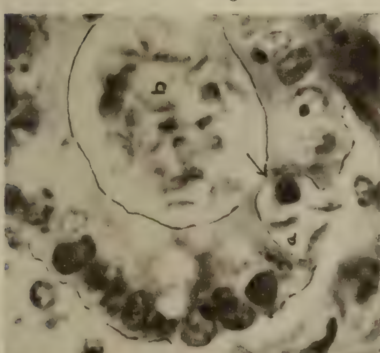

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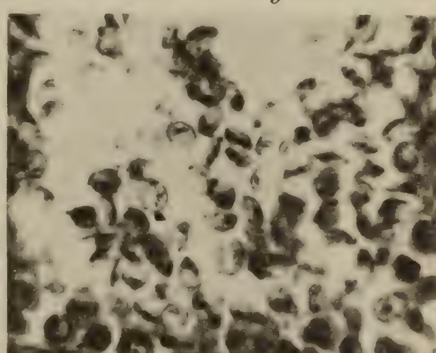

11

ACUTE MERCURY POISONING.*

A PARALLEL HISTOLOGICAL AND CHEMICAL STUDY OF THE RENAL AND HEPATIC TISSUE CHANGES AS COMPARED WITH THE RAPIDITY OF ABSORPTION AND THE AMOUNT OF MERCURY PRESENT IN THE CIRCULATING BLOOD AT THE TIME SUCH CHANGES OCCUR.

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The great majority of those fatal cases of acute mercurial poisoning that do not die within eight to twelve hours after receiving the fatal dose usually succumb on the sixth to eighth day as a result of urinary suppression and its attendant uremia. The accompanying colitis and stomatitis occasionally overshadow this clinical picture. In probably less than ten per cent of the fatal cases, however, do the last-named factors play the more important part in the cause of death. The typical pathological lesions found in kidney and colon in these cases have given rise to much speculation and experimentation. Just how these lesions are produced and just why there should be so marked a selective destructive action on kidney and large bowel has not even at this late day been conclusively proven. The oldest theory as to the manner in which these changes are produced, and the one which at first seems plausible, is the one of elimination. This assumes a direct destructive action on renal epithelium and large bowel mucosa which occurs in the process of eliminating the mercury. This explanation is sponsored in text-books like those of Schmeideberg and Kobert. In an attempt to refute this theory it has been argued that, in its elimination from the body and its concentration in the blood, mercury probably never is in sufficient concentration to do the damage by direct action that we subsequently find at

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necropsy. Other organs in the same individual, liver, salivary gland, and the small bowel, all of which are undoubtedly equally involved in the excretion or absorption of mercury in certainly no less concentrated form, present very mild and frequently no histological changes.

A theory advanced by Kaufman[1] and Heineke,[2] holds that the mercury combinations in the blood have a thromplastic action producing thromboses with a resulting secondary ischemia or infarction. This theory would seem to have been thoroughly eliminated by such workers as Kohan,[3] Sievert,[4] and Priebatsch,[5] the latter having shown that no thrombi are found in the vascular system of the parts involved and the two former having demonstrated that when clotting of the blood is prevented by a previous injection of hirudin no variations result in the histological changes typical of acute mercurialism.

Kunkel[6] believes that a myocardial insufficiency and v. Mering[7] that an accompanying general vasomotor paralysis with its resulting low blood pressure is responsible for the degenerative changes in kidney and bowel; that etiologically these changes resolve themselves into a problem of faulty nutrition due to the extremely low blood pressure. In experimental mercury poisoning, however, Kolb[8] has shown that during the first few days of the intoxication there may be no fall in blood pressure and that more often rise in pressure is experienced, whereas, the characteristic gross and histological changes are at such times present. Giesboeck[9] has found this to be true clinically.

Still another theory, more recent, is that of Elbe,[10] who believes that the anatomical changes are due not to a general, but to a local blood pressure disturbance, in which the mercury present in the blood has a selective action on the vasomotor apparatus of the smaller arterioles of kidneys and large bowel. Its action is to cause a contraction of these vessels to almost complete obturation, thus inhibiting the inflow of blood into the capillary system, resulting in microscopic areas of anemic infarction in the kidney and in hemorrhagic infarction in the large bowel. It has been shown by Rosenheim,[11]

who perfused dog kidneys with defibrinated blood containing mercury salts, that at first one obtains a vasodilation, which is followed shortly by a very marked progressively-increasing vasoconstriction. Natus[12] was able to substantiate this vasoconstricting action of corrosive sublimate on the vessels of pancreas and mesentery. Recent experiments by Weiler[13] would seem to affirm in part the conclusions of Elbe. Just why it should be logical to conclude that the renal and ileocolic vasomotor system is so supersensitive to the action of mercury, whereas a supersensitiveness for the same toxic agent on the part of certain renal cells or colon mucosa is concluded to be impossible, is, however, not quite apparent from the work of Elbe and Weiler.

Flexner and Sweet[14] have shown most conclusively that the changes in the large intestine are the result of the excretory eliminating effort on the part of the colon mucosa, and that the process of elimination is materially handicapped by the repeated re-absorption from the small intestine, of the mercury contained in, and being conveyed from the liver by the bile, in the liver's effort to cast off the toxic substance.

From the foregoing it would seem not unlikely that more than one factor may be operative in the production of the characteristic tissue changes occurring in remote organs following the ingestion of toxic doses of mercury compounds. Thus these changes may be due indirectly to local vasomotor disturbances, or to direct injury of the parenchymal cells by the metal compound. Both factors may act simultaneously, or the action and effect of one may follow closely upon the other. If past investigation is to be of practical value in the future, certain additional problems must be solved. Important among these are the determination of the minimum rate of time in which these lesions can be produced, and a comparative study of any variation in degree of injury following different periods of duration of the intoxication, or any variation in the effect of different, yet supertoxic doses. Parallel to this, chemical studies of the blood should be made to determine the rate of absorption and the amount of mercury

present at the time such changes are being produced. Vlissinger[15] has found that in massive intoxication the intensity of the kidney lesions is influenced to a greater degree by the rapidity of the absorption than it is by the duration of the intoxication. Within the liver he finds the changes progressive, and here the period of duration of the intoxication is of greater import than the intensity of the dose employed. Minor liver changes are found within an hour, these and others become more manifest during the next twenty-four to one hundred and twenty hours. Lesions in the large intestine are slower to appear and do not begin until some time after changes are found in the liver, usually not before three or more hours after the onset of the intoxication. Elbe[10] has found that loss of the nuclei of the renal cells of convoluted tubules occurs after nine hours, and that necrosis of the mucosa of the tips of the folds in the cecum begins with an edema within six hours and is complete at twelve hours. Whatever the mechanism by which these changes are produced, it is evident that locally tissue change in kidney, liver, or large intestine, occurs only after the transport of the toxic substance by the blood to these organs. While actual necrosis may not be evident for many hours, certain irreparable degenerative changes become manifest much sooner. A study of these changes parallelled by a chemical study of the blood at the time these changes were being produced was the objective of the following experiments:

In all, the kidneys, liver, and large intestine of fourteen dogs, poisoned by mercuric chloride were studied and blood analyses were made in each instance. The corrosive sublimate was administered in a slightly acid water solution on an empty stomach per stomach tube. Partial or complete anesthesia was previously produced by the subcutaneous injection of chloretone dissolved in olive oil. Per kilo of weight .7 gram of chloretone was injected several or more hours prior to the administration of the sublimate. In some instances the chloretone had to be supplemented by ether to

prevent vomiting. The dogs were bled to death from the femoral artery and all of the blood reserved for chemical and microscopical examination. In a few instances death occurred before blood could be obtained from the artery or before the bleeding could be completed. At such times blood was obtained by immediate cardiosection. With the exception of Dogs 1 and 2 separate chemical examinations were made for each dog, of whole blood, washed corpuscles, and of the plasma and washings from these latter corpuscles. The corpuscles to be washed were received in sodium citrate solution and washed six to seven times with twice their volume of Ringer's solution by centrifugalizing at 2,000 revolutions for fifteen minutes. Several different qualitative and quantitative chemical methods were utilized in the blood analyses. These results are tabulated in the Chart. Plasma and washings from the corpuscles of Dogs 4, 7, 9, and 11, were examined by Salkowski's method as modified by Perlstein and Abelin.[16] Whole blood from Dogs 10, 12, 13, 14, and washed corpuscles from the blood of Dogs 3, 4, 5, 6, 8, 10, 11, 12, 13, 14, were examined by Ludwig's method.[17] In the analyses of the plasma and washings from the blood of Dogs 3, 5, 6, 8, 10, 12, 13, 14, the determinations were made electrolytically. Here the mercury was separated by the method of Perlstein and Abelin and deposited electrolytically on gold foil, using platinum and gold electrodes. The mercury was removed from the gold electrode by sublimation in glass tubing, and the weight of the sublimed metallic mercury obtained direct. Determinations for whole blood from Dogs 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, and for washed corpuscles from the blood of Dogs 7, 9, were made according to Fresenius and v. Babe's method of digestion, the mercury being precipitated as the sulphide and then reduced by hydrogen peroxide, and its weight determined as calomel, according to the method of Rose.[18]

That these methods are subject to error will be seen from the Chart, in which it will be noted that in five instances (Dogs 8, 9, 10, 13, 14), mercury, though not found in the

whole blood, was subsequently isolated by one or the other methods from the washed corpuscles or the plasma and washings of blood from the same animal. This was probably due to the fact that the determinations in the latter instance were made from a quantity of blood considerably larger than that used in the analyses of the whole blood. This is further born out by the fact that in four of the five instances only a trace of mercury was found; so little that it could not be estimated quantitatively. However, in Dog 12, a weighable quantity of mercury was obtained from the whole blood despite the fact that the washings and corpuscles, each separately from a much larger quantity of blood, failed to reveal a trace of the metal. Similar results were obtained in Dog 7, though here the whole blood contained but a trace of mercury. In Dog 4, mercury was found in the whole blood and in the washed corpuscles, but not in the washings. In Dogs 3, 5, 8, mercury was present in the whole blood and in the washings while the washed corpuscles were minus any trace of the metal. In Dog 6 mercury was found in all three determinations.

Bichloride of mercury was administered to these dogs in doses varying in amount from .024 gram to .5 gram per kilo of weight. Dogs weighing from three to sixteen kilos were used. From the chemical examinations the great rapidity with which the mercury was absorbed from the stomach as determined by its presence in the blood will be noted. As will be seen from the Chart, with doses as low as .024 per kilo of dog weight, mercury could be detected qualitatively in the blood within ten minutes after it was administered. With larger doses (.25 to .5 gram per kilo of dog weight) quantitative determinations were possible in less time. As estimated for Dog 2, three minutes after the ingestion of .5 gram of mercuric chloride by the mouth there was present in her total blood volume .00035 gram of metallic mercury, or the equivalent of .00041 gram of mercuric chloride.

Almkvist,[19] Weiler[13] and others have noted the presence of small black irregular granules in the tissues undergoing

degenerative changes secondary to mercury poisoning. These are held by Almkvist to be depositions of mercuric sulphide. This is denied by Weiler, who maintains that hydrogen sulphide is at no time, during life, present in the blood in sufficient concentration to precipitate mercury from its blood solution. Whatever their exact nature, these granules are frequently encountered. Usually they have been judged some artefact due to a fixing or staining method. They resemble morphologically the black granules so often found in tissues fixed in Zenker's solution, artefacts due to insufficient washing, resulting in incomplete removal of the mercury carried in by the fixing solution. It is interesting to note that such granules were present not only in the tissues studied, but were also found microscopically in whole blood among the washed corpuscles, and in the washings from these corpuscles, in each of the dogs studied in this series. They appeared as free granules, never present in the erythrocytes. Frequently a white corpuscle was found to contain a number of minute black granules within its cytoplasm and this became more evident and almost a constant finding when the unstained film was previously immersed for a moment in a distilled water solution of hydrogen sulphide. These findings are interesting when compared with those of Bissel[20] who cites the presence of mercury in the leucocytes of human beings poisoned with mercuric chloride.

At immediate necropsy following the death of the dog, portions of both kidneys, pieces from various lobes of the liver, a part of the stomach wall, a piece each of sigmoid and colon were placed in ten per cent formalin. Each block of material selected from these tissues was then divided in two, and one portion was embedded in celloidin and its sections stained with hematoxylin and eosin. Frozen sections were made of the other piece and sections stained with Sudan III, and others unstained were examined after passing the latter through an acidulated watery solution of hydrogen sulphide. The frozen and blocked sections were studied at the same time. The pathological changes in the organs of the various dogs were quite uniform in character, though the

degree of injury as evidenced by the histological alterations, varied considerably. These changes, as noted in the protocol of Dog 10, will be detailed here. Those of the remaining dogs are briefly summarized in the Chart.

Dog 10. Microscopical changes. — Kidney: Frozen sections unstained and H_2S treated: Marked cloudy swelling and hydropic degeneration of epithelium of those tubuli contorti located immediately subcapsular. The intertubular capillaries here can scarcely be discerned; they are collapsed or contracted and contain little or no blood. The epithelium of the convoluted tubules of the deeper cortical regions is involved to a lesser degree. Here there is a very moderate parenchymatous degeneration and the nuclei and the basal striations are quite well preserved in most instances. The capillaries of the glomeruli are distended. The glomerular epithelium is somewhat swollen and the space between Bowman's capsule and the corpuscle is decreased in width. Approximately one-third of all the ascending limbs of the loops of Henle present a most marked change in their epithelium. The cells are swollen, and their cytoplasm contains large droplets of a highly refractile substance which causes the lumen boundary of the cells to bulge, until opposite cells are frequently in contact with one another. The cell boundaries are indistinct and it is difficult to make out the nuclei. Usually all the cells of the ascending limb of the same tubule are equally and uniformly involved. Other and immediately adjacent tubules sometimes appear uninjured. The cells of the descending limbs and those of the collecting tubules are intact with the exception of an occasional group of epithelium in some of the larger end-collecting tubules. Rarely these are found to contain a few minute refractile droplets in their cytoplasm. The intertubular capillaries in the Malpighian pyramids are distended. In the larger renal arterioles are frequently seen black irregular granules varying in size from one-half to twice the size of an erythrocyte — these are extracellular. Occasionally a leucocyte appears laden with a number of minute, but otherwise similar granules in its cytoplasm. The interstitial areolar tissue about these vessels appears edematous and frequently in the lymph spaces immediately adjacent to the vessel are seen a group of the larger granules. Only rarely, and then only singly, are such granules found in the glomeruli capillaries. Sections stained with Sudan III indicate the refractile droplets noted above to be fat. Rarely some of the cells of the more severely altered subcapsular convoluted tubules are seen to contain a small number of minute droplets staining with Sudan III. Within the cytoplasm of the renal cells presenting these degenerations were frequently found minute granules resembling the black granules cited above. Hematoxylin and eosin-stained sections presented no additional changes.

Liver: Frozen sections, H_2S treated and stained with Sudan III: Great numbers of large black irregular granules (resembling those found

in kidney sections) are seen in a number of the larger portal veins and scattered adjacent to the walls of these veins in the edematous connective tissue of the periportal fields. Occasionally smaller granules are seen in the capillaries between the hepatic cords. There is a marked parenchymatous degeneration of the hepatic cells of the central zone of the acinus, though most of the cells of the entire acinus are involved. These changes become usually less marked at the periphery, where not infrequently the outer zone of cells appears intact. The capillaries between the liver cords are not dilated and contain little blood. None of the black granules referred to are found in the parenchymal cells, though frequently a few very minute black granules appear to be located in a Kupfer's cell. Such granules are also infrequently seen within the cytoplasm of the epithelium of some of the larger bile ducts, and are occasionally free in the lumen of these ducts. No fatty changes of a degenerative character are seen in the sections treated with Sudan III.

Stomach: Frozen sections, unstained, H_2S treated, and Sudan III stained: The surface epithelium appears unchanged. The sub-mucosa is markedly edematous. Within the larger vessels of the sub-mucosa are seen black granules similar to those described in sections of liver and kidney. The interglandular capillaries are contracted. Sudan III sections present no fatty changes. Sections stained with hematoxylin and eosin reveal an epithelium, which appears normal despite the fact that the tissue was chosen from an area of stomach wall, the mucosa of which macroscopically had the typical gray-cooked appearance. Apparently the cells had been killed and fixed simultaneously and instantly by the large amount of sublimate administered.

Colon and sigmoid: Frozen sections, unstained, H_2S and Sudan III treated: Here, too, the characteristic black granules are seen in the larger vessels of the sub-mucosa. They are less frequently seen than they were in stomach. No fatty changes are noted in the Sudan III sections. Hematoxylin and eosin stains reveal no changes other than a very slight increase in the number of goblet cells.

As will be seen from the Chart, Dog 10 had received his chloretone three hours prior to the administration of the mercury. Additional ether anesthesia was necessary to prevent vomiting. He was killed one hour and thirty-four minutes after the administration of .055 gram of mercuric chloride per kilo of weight. His protocol was chosen here because he had been under the influence of chloretone but a short time. I mention this because it was noted in two controls which were given the same dose of chloretone and killed after twenty-four hours, that the epithelium of the

tubules of a number of the ascending limbs of Henle presented a fatty degeneration of a moderate degree. It is interesting to note that the fatty changes in the kidney, as will be seen from the Chart, appear most marked in those dogs which had been under the influence of chloretone for twenty-four-hour periods. That these fatty changes are also characteristic of mercury poisoning, even to the degree noted above, I have since satisfied myself in another series of fourteen dogs, none of which received chloretone. They have been noted also by practically all who have studied experimental mercury poisoning.

It will be seen from the Chart that where massive doses of mercuric chloride acted for a short time (Dogs 1 and 2), the kidney changes were quite marked and that changes were present in the liver in a moderate degree. Smaller doses acting for a short time (Dogs 3, 5, and 13), produced less marked kidney lesions, with moderate but increasing hepatic changes. In one instance, Dog 13, where the renal changes were the least severe, the hepatic degeneration was most marked. Apparently not in accordance with the results of Vlissinger,[15] it will be seen that in my series smaller doses acting over longer periods of time produce kidney lesions equally as severe as those found where larger doses act for shorter intervals. This is seen in Dogs 6, 7, 8, 10, 11, 12. Here, too, the hepatic lesions are much advanced in degree and occasionally a marked involvement of bile duct epithelium is found. A relatively small dose which in Dog 9 caused almost no renal changes and only slight hepatic alterations, produced in Dog 8, acting over a period of time three times as long as the duration in Dog 9, most marked renal and hepatic changes. In all of the above instances it was possible to isolate mercury from the circulating blood. It will, however, be seen that the degree of renal and hepatic change does not vary directly as does the quantitative chemical determination. That severe parenchymatous degenerations should appear within periods of time as short as three minutes (Dog 2), is not incompatible with

our findings in other severe toxemias. The rapidity with which more advanced retrogressive change, fatty degeneration, manifested itself, is, however, rather unusual. We are taught that pathologically cloudy swelling may merge retrogressively by a very short step into fatty degeneration, and that fatty degeneration retrogressively by another short step becomes necrosis. It is further believed that a cell in a state of cloudy swelling may possibly recover and again become normal, but that a cell whose protoplasm has undergone a chemical deconstitution — as we suppose the condition to be in fatty degeneration — is hopelessly lost and ultimately dies. It is not strange then if such degenerative changes can occur in so short a period of time that our therapeutic efforts in so many cases of acute mercuric poisoning count for naught.

CONCLUSIONS.

I. Mercury can be detected chemically, frequently quantitatively, in the blood of animals poisoned with mercuric chloride, within a very few minutes after it is administered.

II. Degenerative changes, leading to individual cell death, take place rapidly in the kidney and occur simultaneously with the presence of mercury in the circulating blood.

III. In massive intoxication immediate renal changes vary with the size of the dose. Hepatic changes vary as the duration of the intoxication. In smaller doses renal changes vary as the duration of the intoxication.

IV. The degree of renal or hepatic degeneration does not necessarily vary directly as does the amount of mercury contained in the circulating blood.

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Dog.	Weight and Sex.	›scopical Findings.	Anesthetic.
1	Female. 3.2 k.	oops marked; cloudy swelling sub- Liver, cloudy swelling parenchymal f acinus.	E. 24 hours. Died while being bled.
2	Female. 4 k.	Dog 1.	E. 24 hours earlier. F.
3	Male. 16 k.	n Dogs 1 and 2. Great number l portal vessels.	E. 24 hours earlier.
4	Female 8.4 k.	. Liver, central cloudy swelling	E. 24 hours earlier. F.
5	Male. 12 k.	, no fatty degeneration. Liver	E. 2 hours earlier. F.
6	Male. 12 k.	ty changes quite marked. Liver, n center of acini.	E. 24 hours earlier.
7	Femal	ry many black granules in vessels. marked	E. 24 hours earlier. F.

CHART.

Dog.	Weight and Sex.	Total Amount $HgCl_2$ Administered. Dose Per Kilo.	Whole Blood Determinations (Duration of Intoxication. Time of Bleeding.)	Estimations for Washed Corpuscles.	Estimations for Plasma Plus Washings.	Principal Microscopical Findings.	Anesthetic.
1	Female 3.2 k.	1.5 grams. 0.5 gram per k.	A. 137 grams. 5 minutes. B. Hg present trace. C. F. and v. B. — R.			Fatty degeneration, ascending loops marked; cloudy swelling subcapsular convoluted tubules. Liver, cloudy swelling parenchymal cells most marked in center of acinus.	E. 24 hours. Died while being bled.
2	Female 4 k.	0.5 gram. 0.125 gram per k.	A. 168 grams. 3 minutes. B. 0.0002 gram Hg. C. F. and v. B. — R.			Kidney and liver findings as in Dog 1.	E. 24 hours earlier. F.
3	Male 16 k.	1.0 gram. 0.065 gram per k.	A. 154 grams. 12 minutes. B. Hg present trace. C. F. and v. B. — R.	D. 382 grams. 5 minutes. B. Hg negative. C. Zn. — R.	B. Hg present trace. C. Electrolytic.	Changes in kidney and liver as in Dogs 1 and 2. Great number of black granules in renal and portal vessels.	E. 24 hours earlier
4	Female. 8.4 k.	1.0 gram. 0.12 gram per k.	A. 150 grams. 18 minutes. B. Hg present trace. C. F. and v. B. — R.	D. 131 grams. 15 minutes. B. 0.0002 gram Hg. C. Zn.	B. Hg negative. C. Salkowski.	Kidney changes as in Dog 1. Liver, central cloudy swelling more marked.	E. 24 hours earlier. F.
5	Male. 12 k.	1.0 gram. 0.083 gram per k.	A. 183 grams. 35 minutes. B. 0.0002 gram Hg C. F. and v. B. — R.	D. 291 grams. 30 minutes. B. Hg negative. C. Zn. — R.	B. 0.0001 gram Hg. C. Electrolytic.	Very little change in kidney, no fatty degeneration. Liver changes very moderate.	E. 2 hours earlier. F.
6	Male. 12 k.	1.0 gram. 0.083 gram per k.	A. 92 grams. 52 minutes. B. Hg present trace. C. F. and v. B. — R.	D. 190 grams. 48 minutes. B. Hg present trace. C. Zn. — R.	B. Hg present trace. C. Electrolytic.	Kidney changes as in Dog 1, fatty changes quite marked. Liver, cloudy swelling most marked in center of acini.	E. 24 hours earlier.
7	Female. 7 k.	1.0 gram. 0.143 gram per k.	A. 60 grams. 60 minutes. B. Hg present trace. C. F. and v. B. — R.	D. 180 grams. 60 minutes. B. Hg negative. C. F. and v. B.	B. Hg negative. C. Salkowski.	Kidney changes as in Dog 1; very many black granules in vessels. Liver, central cloudy swelling marked.	E. 24 hours earlier. F.
8	Female. 11.5 k.	0.5 gram. 0.043 gram per k.	A. 109 grams. 142 minutes. B. Hg present trace. C. F. and v. B. — R.	D. 265 grams. 142 minutes. B. Hg negative. C. Zn. — R.	B. Hg present trace. C. Electrolytic.	Kidney, fatty degeneration in ascending limbs Henle most marked. Black granules in vessels abundant. Liver, central cloudy swelling, fatty degeneration epithelium larger bile ducts.	E. 3 hours earlier. F.
9	Female 11.5 k.	0.5 gram. 0.043 gram per k.	A. 91 grams. 45 minutes. B. Hg negative. C. F. and v. B. — R.	D. 162 grams. 45 minutes. B. Hg negative. C. F. and v. B. — R.	B. Hg present trace. C. Salkowski.	Kidney, very little change in any portion. Black granules prominent in vessels. Liver, very slight cloudy swelling.	E. 3 hours earlier. F.
10	Female. 9 k.	0.5 gram. 0.055 gram per k.	A. 126 grams. 94 minutes. B. Hg negative. C. Zn. — R.	D. 201 grams. 89 minutes. B. Hg negative. C. Zn. — R.	B. Hg present trace. C. Electrolytic.	Kidney changes as in Dogs 1 and 2, the fatty degeneration quite marked. Liver changes as in Dogs 1 and 2.	E. 3 hours earlier. F.
11	Female. 10.5 k.	0.25 gram. 0.024 gram per k.	A. 168 grams. 145 minutes. B. Hg negative. C. F. and v. B. — R.	D. 141 grams. 145 minutes. B. 0.0002 gram Hg. C. Zn. — R.	B. Hg negative. C. Salkowski.	Kidney, fatty degeneration and black granules very prominent. Liver, cloudy swelling of parenchyma and fatty degeneration of bile duct epithelium.	E. 24 hours earlier. F.
12	Female. 10 k.	0.25 gram. 0.025 gram per k.	A. 163 grams. 170 minutes. B. 0.0001 gram Hg. C. Zn. — R.	D. 329 grams. 170 minutes. B. Hg negative. C. Zn. — R.	B. Hg negative. C. Electrolytic.	Kidney, changes as in Dog 1, fatty degeneration most marked. Liver, general cloudy swelling and slight central fatty degeneration.	E. 24 hours earlier. F.
13	Male. 10.5 k.	0.25 gram. 0.024 gram per k.	A. 76 grams. 10 minutes. B. Hg negative. C. Zn. — R.	D. 205 grams. 10 minutes. B. Hg present trace C. Zn. — R.	B. Hg present trace. C. Electrolytic.	Kidney, marked cloudy swelling convoluted tubules, practically no fatty changes. Liver, cloudy swelling and central fatty degeneration. Black granules abundant.	E. 24 hours earlier. F.
14	Female 10.5 k.	0.5 gram. 0.048 gram per k.	A. 88 grams. 30 minutes. B. Hg negative. C. Zn. — R.	D. 155 grams. 30 minutes. B. Hg present trace. C. Zn. — R.	B. Hg present trace. C. Electrolytic.	Kidney, moderate cloudy swelling, moderate fatty changes. Black granules abundant. Liver, central cloudy swelling, slight amount of fatty degeneration in peripheral zones.	E. 24 hours earlier. F.

A. Amount of blood analyzed and time at which this blood was taken.
B. Results of analyses for mercury.
C. Chemical tests employed.
D. Amount of whole blood from which corpuscles analyzed were obtained; time at which blood was taken.
Note. — The plasma and washings are from the same whole blood from which the washed corpuscles were obtained. In most instances 500 cubic centimeters of the mixture of plasma and washings were analyzed. Unfortunately, due to an error, no record was made of the total volume of plasma plus washings.
E. Chloretone, time of administration.
F. Ether, supplementary anesthesia only at the time of the experiment in cases in which the action of the chloretone proved insufficient.

THE CLASSIFICATION OF STREPTOCOCCI.*

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The classification of the streptococci in spite of the extensive study that has been devoted to the subject still remains in a state of confusion. If one searches for an explanation of the diverse and inconsistent results reported in the literature, it at once becomes evident that diversity of method and technic is responsible in large measure for this confusion. There has been little attempt to simplify and standardize the various methods in use or to correlate the various schemes of classification that have been proposed. Instead, the tendency has been toward the multiplication of methods and the introduction of an ever increasing number of classifications.

It is the purpose of this paper to attempt a correlation of previously reported work and to report the study of one hundred and sixty-one strains of streptococci isolated from human sources, in order that a practicable classification of the streptococci for clinical purposes may be arrived at.

Critical review. — Of the various methods of classification, the two which have been used most extensively and which have proved the most satisfactory are the blood-agar plate method introduced by Schottmüller in 1903, and the carbohydrate fermentation method proposed by Gordon in the same year. One or the other of these methods, or in rare instances a combination of the two, has formed the basis of most subsequent classifications. A primary division of the streptococci by means of the blood-agar plate method seems to me essential for reasons which will be developed. Following this primary division further subdivisions by means of a limited number of carbohydrate fermentation tests can be carried out and is of some practical value. It is upon this basis that the classification to be proposed is founded.

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Blood-agar plate method. — Schottmüller recognized three types of streptococci by the characteristics of the colonies on blood-agar plates. (1) *Streptococcus longus pathogenes seu erysipelatos*, which produced a wide clear zone of hemolysis about the colony. (2) *Streptococcus mitior seu viridans*, which produced green colonies occasionally with a narrow zone of hemolysis in which, however, there was no destruction of the red blood cells such as occurred with the true hemolytic streptococci of group (1). (3) *Streptococcus mucosus*, which produced large mucoid gray or bluish green colonies. This group is undoubtedly the group of organisms at present classified as *Pneumococcus mucosus*, being bile soluble, and need not be considered further in this connection.

Schottmüller called attention to several important points in technic to be observed in the use of this method and they need to be emphasized. A sufficient amount of blood must be mixed with the agar, and the mixture must be even or the results will not be clear cut. He advocated the use of forty per cent of defibrinated blood. Such an amount is unnecessarily large, however, and I have found ten per cent thoroughly satisfactory, while Holman has had equally favorable results with five per cent. An even mixture of the blood and agar I have found to be absolutely essential and with a little care this can be obtained easily. Another important point brought out by Schottmüller is that the colonies must be well separated and this cannot be too strongly emphasized. On plates with numerous closely aggregated colonies the results are often confusing. For this reason surface streaking as it is commonly done is often inadequate, as Holman has pointed out. And lastly, colonies of Streptococcus viridans in addition to the green color produced occasionally show a narrow zone of hemolysis. This has apparently been a source of confusion to some authors, but in the writer's experience, if the points in technic mentioned above are carefully adhered to, the two types of streptococci are readily differentiated and are quite distinct.

Subsequent studies of streptococci by the blood-agar plate method have in the main thoroughly substantiated its reliability and practicability as a means of primary differentiation into two large groups. In 1904 Rosenow independently called attention to the fact that streptococci produced hemolysis on blood agar while pneumococci produced a green color with occasionally a narrow zone of hemolysis. That strains of Streptococcus viridans were classed with pneumococci by him at that time is now evident and needs no further comment. Fränkel, Ruediger, Mandelbaum, Baumann, Le Blanc, Rolly, Smith and Brown, Holman, and many others have used the blood-agar plate method and found it satisfactory. Mandelbaum, Le Blanc, and Rolly have pointed out that in addition to the hemolytic and green-producing, there is a third group of streptococci which produces neither hemolysis or methemoglobin and have proposed the name Streptococcus saprophyticus for this type. Lyall, using sheep corpuscles instead of blood-agar plates to determine the action of streptococci on blood, has divided them into three groups. (1) hemolytic, (2) methemoglobin producers, and (3) indifferent. He states, however, that strains which fail to produce methemoglobin when tested against sheep corpuscles often grow as green colonies on blood-agar plates. I have tested a considerable number of strains by both methods and found this to be true. Holman, while recognizing the occurrence of indifferent strains, feels that they should be classified with Streptococcus viridans. I am in accord with this view because the two groups show the same types of fermentation reactions, are derived from the same sources, are in general similar in morphology, and are of comparable pathogenicity. Smith and Brown have used blood-agar plates as a means of differentiation and have described two types, α and β, which correspond to Streptococcus viridans and the hemolytic streptococci respectively so far as can be judged from their descriptions.

Hopkins and Lang on the other hand have found the blood-agar plate method unsatisfactory and state that all gradations of hemolysis exist between the viridans type and

strains producing a wide hemolytic zone. Their results, I believe, may be explained by the fact that their observations often extended over a period of three days' incubation rather than being made at the end of twenty-four hours, that the blood was not always evenly mixed with the agar, and since they used the surface-streak method the colonies may not have been sufficiently separated.

Ruediger has explained the formation of green colonies on blood agar as due to acid production, because hemolytic streptococci when grown on blood agar containing a carbohydrate fermented by the strain failed to produce hemolysis, but rather a green color. His findings have been substantiated by others, but I agree with Holman in believing that his explanation is incorrect. It has been shown by Lyall that the presence of dextrose inhibits hemolysis production by streptococci. This is probably due to the inhibition of growth by the acidity produced, as the majority of hemolytic streptococci are particularly susceptible to an increased acidity. Furthermore, the degree of acidity produced by the fermentation of the carbohydrate in Ruediger's media was probably sufficient to change oxyhemoglobin to methemoglobin, thus explaining the green coloration produced by the hemolytic streptococci when grown under these conditions. That methemoglobin formation by Streptococcus viridans is not due to acid production has been shown by the author, for if an ascitic bouillon culture of Streptococcus viridans be incubated with a suspension of washed sheep corpuscles with the addition of a mixture of primary and secondary potassium phosphates so that neutrality is maintained, methemoglobin formation is not inhibited, but is actually accelerated.

That the narrow zone of clearing sometimes occurring around colonies of Streptococcus viridans is not a true hemolysis has been shown by Mandelbaum and Le Blanc, who found that the red blood corpuscles in the clear zone did not lose their structure as they do in the wide zone of clearing occurring about colonies of true hemolytic streptococci.

Holman believes that the methemoglobin formed by Streptococcus viridans is liberated to some extent from the cells, and is stored in the colony, rendering the altered cells more liable to spontaneous hemolysis. I am in accord with these opinions, and do not believe that the narrow zone of clearing that sometimes appears about Streptococcus viridans colonies is due to a true hemolytic function of this group of streptococci. This view I have substantiated by numerous experiments with many strains of Streptococcus viridans, in which ascitic bouillon cultures have been incubated with washed sheep corpuscles for two hours at 37° C. Under these conditions methemoglobin formation has always occurred, and no trace of hemolysis has been demonstrable. On the other hand, hemolytic streptococci under the same conditions have consistently caused complete hemolysis without methemoglobin formation in from ten to thirty minutes.

In summary it may be said: (1) the blood-agar plate method of differentiating streptococci has proved reliable and practicable in the hands of most observers; (2) inconsistency in results may be obviated by attention to the following points in technic — at least five per cent of defibrinated blood should be used, the blood and agar must be evenly mixed, the colonies must be well separated, and readings should be made at the end of twenty-four hours' incubation; (3) by this method two distinct types of streptococci may be differentiated by means of characteristic metabolic functions — (a) hemolytic streptococci which produce an active hemolysin without methemoglobin formation; (b) green-producing streptococci which change oxyhemoglobin to methemoglobin without the production of a true hemolysin.

Carbohydrate fermentation method. — The differentiation of streptococci by the carbohydrate fermentation method, introduced by Gordon in 1903, has been widely used. Careful analysis of the results obtained, while at first appearing rather confusing, nevertheless reveals the fact that most of the inconsistencies that appear on the surface are in reality

due, firstly to failure to recognize that a primary division into hemolytic and methemoglobin-producing groups is essential for proper classification, inasmuch as the same fermentation type reactions occur in both groups, and secondly to variations in technic, particularly with respect to the media used and the indicator selected to determine a positive reaction. A review of the more important communications upon this method will serve to emphasize these points.

Gordon was the first to attempt classification of the streptococci by means of fermentation reactions. After preliminary tests he selected seven substances as being of differential value; namely, lactose, saccharose, raffinose, inulin, salicin, coniferin, and mannite. In addition he studied coagulation of milk and reduction of neutral red broth in anærobic culture. Using faintly alkaline sugar-free broth tinted with litmus, to which the substance to be tested was added, he applied these tests to 300 strains of streptococci from twenty-two samples of saliva and found forty-eight types, ten of which contained ten or more strains.

Andrewes and Horder in 1906 reported an extensive study of streptococci by Gordon's method, and offered the first definite classification. They tested 228 strains isolated largely from pathological sources, and tabulated the 972 strains previously reported by Gordon and by Houston, making a total of 1,200 strains. In addition to Gordon's tests they considered growth on gelatin at 20° C., type of chain formation, and pathogenicity for mice of differential value. Although they stated that most highly pathogenic strains were hemolytic, it is not evident that they used the blood-agar plate method as a means of differentiation. Since their classification has formed the basis of most subsequent ones, it seems of advantage to present their results in tabular form (Table I.):

TABLE I.

Andrewes and Horder's types of streptococci.

	Name.	Milk Clot.	Neutral Red.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Coniferin.	Mannite.	Growth on Gelatin at 20° C.	Morphology.	Pathogenicity for Mice.
A	Str. equinus . . .	—	—	±	—	—	—	±	±	—	—	Medius.	—
B	" mitis	—	±	+	+	—	—	±	±	—	+	Brevis.	—
C	" pyogenes . . .	—	—	+	+	—	—	±	—	—	+	Longus.	+
D	" salivarius . .	+	±	+	+	±	—	—	—	—	±	Brevis.	—
E	" anginosus . .	+	±	+	+	±	—	—	—	—	±	Longus.	+
F	" fecalis	+	+	+	+	—	—	+	+	+	+	Brevis.	—
G	Pneumococcus . .	±	—	+	+	+	±	—	—	—	—	Brevis.	+

Although Andrewes and Horder's classification has much to recommend it, it is nevertheless in many ways unsatisfactory. The numerous ± signs indicate that they have encountered numerous variants from the type reactions, and that a given strain is placed under one type or another according to individual judgment. Careful analysis of the numerous variant strains shows that Andrewes and Horder based this judgment on several factors, namely, the source of the organism, the type of chain formation, the characteristics of the growth in broth, and the pathogenicity for mice.

While it is well recognized that the majority of highly pathogenic strains are hemolytic, are long chained, and grow as a sediment in broth, it is nevertheless true that methemoglobin-producing strains are not infrequently encountered which are pathogenic, or grow as a sediment in bouillon with long-chain formation, and possess the same type-fermentation reactions as the hemolytic strains. It is evident then that confusion must arise if primary differentiation by the blood-agar plate method is not used. Pathogenicity, the characteristics of growth in bouillon, and the type of chain formation are notably inconstant, and bear no consistent relation to the blood or fermentation reactions.

For these reasons they are not to be recommended as a means of differentiation.

The number of tests advocated by Andrewes and Horder is too large for practical purposes and results in a multiplicity of variants from the main types, thereby tending to confuse rather than to clarify the results. The use of milk adds nothing that is not obtained by the use of lactose. The failure to clot milk by strains which ferment lactose simply indicates a low acid tolerance by such strains. The fact that Streptococcus pyogenes (Andrewes and Horder), which is notably susceptible to an increased acidity gives this result emphasizes the point. The reduction of neutral red is of little differential value, and most observers have found its use unsatisfactory. Coniferin fermentation largely parallels the results with salicin. Growth on gelatin of recently isolated strains is unsatisfactory and it is a readily acquired property after cultivation on artificial media. For these reasons it has seemed to me advisable to discard these tests in analyzing previously reported work and to retain only saccharose, lactose, raffinose, inulin, salicin, and mannite for this purpose. If this is done Andrewes and Horder's types Streptococcus mitis and Streptococcus pyogenes show the same group reactions, and Streptococcus anginosus corresponds to Streptococcus salivarius. These groups, however, can be readily separated by the blood-agar plate method, Streptococcus pyogenes and Streptococcus anginosus being hemolytic, while Streptococcus mitis and Streptococcus salivarius are methemoglobin producers.

Gordon later reviewed the work of Andrewes and Horder and accepted their classification. He reported the study of one hundred and fifty-five strains isolated from the throat in cases of scarlet fever. Streptococcus pyogenes was the predominating type.

Further studies of streptococci with the use of Gordon's technic have been reported by Houston, Cumpston, Buerger, and Beattie and Yates. None of these authors used the blood-agar plate method, so that their results are difficult to

analyze. Their findings, however, are in the main similar to those of Andrewes and Horder.

Buerger was the first to suggest that variation in results might be due to lack of favorable conditions for growth, a point enlarged upon by subsequent authors. To obviate this he added ascitic fluid to the broth and thereby obtained consistently good growth and constant results.

Winslow and Palmer in 1910 reported the study of one hundred and sixteen strains of streptococci from human feces, one hundred strains from horse feces, and eighty-six strains from cow feces. Believing that litmus was an unsatisfactory indicator of positive fermentation, they advocated the use of the titration method with phenolphthalein as an indicator. They titrated the acidity produced after three days' incubation in meat-extract sugar broth, the acidity of control tubes of uninoculated broth being subtracted from the result. Strains producing 1.2 per cent acidity or more were considered as fermenting. Dextrose, lactose, raffinose, and mannite were considered of differential value. No hemolytic tests were performed, but the source of the strains suggests that they were of the *viridans* type. They offered no definite classification and the comparative value of their results is considerably vitiated by the fact that they tested many strains from single specimens of feces.

The titration method has been used with slight variations by many subsequent workers. Stowell and Hilliard, and Stowell, Hilliard, and Schlesinger reported studies of throat and milk streptococci, but arrived at no definite classification. Fuller and Armstrong in a study of fecal streptococci confirmed the results of previous workers on this group of organisms.

Broadhurst has made an extensive study of streptococci by the fermentation method. She used saccharose, lactose, salicin, raffinose, mannite, and inulin broth, determining positive fermentation by the titration method of Winslow and Palmer. She, however, considered 1.5 per cent acidity or more as indicative of fermentation. She emphasized the point first brought out by Buerger that good growth was

essential for accurate results and showed by parallel tests with meat-infusion and meat-extract broths that the results with meat-infusion broth were always higher than with meat-extract broth. She considered that this factor and the choice of different indicators was responsible in large measure for many of the discrepancies appearing among the results of different workers. In all she has reported on seven hundred and seventy-six strains and has presented a classification that is modeled on that of Andrewes and Horder without materially improving upon it.

Floyd and Wolbach have reported on two hundred and forty-seven strains from human disease sources. They recognized the value of primary differentiation by the blood-agar plate method and submitted all their strains to this test. For study of the fermentation reactions they employed dextrose, lactose, mannite, raffinose, inulin, and saccharose neutral red serum water. They found a surprisingly large number of non-fermenting strains and in this respect their results are at variance with those of other workers.

Lyall in 1914, after study of two hundred and sixty-three strains of streptococci and pneumococci, presented a classification dependent on a combination of the fermentation reactions and the change produced when eighteen-hour ascitic bouillon cultures were tested against washed sheep corpuscles at 37° C. for one hour. He used litmus serum water to which the test substance was added and considered salicin, raffinose, mannite, and inulin of differential value. Results were recorded after seven days' incubation. He advocated the fermentation of inulin as a means of primary differentiation into pneumococcus and streptococcus groups. A primary division of the streptococci into three groups was made by means of the sheep-corpuscle test: (1) hemolytic, (2) methemoglobin producers, and (3) indifferent. The hemolytic group was characterized by salicin fermentation and designated Streptococcus pyogenes. The methemoglobin-producing group was characterized by the fermentation of raffinose (salicin$\pm$) and included Streptococcus

viridans and variants. The indifferent group was characterized by salicin fermentation (raffinose$\pm$) and included Streptococcus fecalis and other streptococci. Lyall's classification, while excellent in that it recognizes the value of division by the blood test, leaves much to be desired. Primary division into pneumococcus and streptococcus groups by means of inulin fermentation is inferior to the Neufeld bile test, as the latter is constant while a not inconsiderable number of streptococci that ferment inulin are encountered. The separation of non-hemolytic streptococci into methemoglobin producing and indifferent groups is not advantageous for reasons that have been stated above and they are better classed together under one head — Streptococcus viridans. Raffinose fermentation is not a constant characteristic of methemoglobin producers and mannite should be included as the characteristic fermentation reaction of Streptococcus fecalis. Finally, the omission of lactose fails to provide for the group of non-lactose fermenters — Streptococcus equinus of Andrewes and Horder, which nearly all workers have encountered in considerable numbers.

Smith and Brown in a comprehensive study of streptococci from milk-borne epidemics of septic sore throat have brought out several important points, though not attempting a definite classification other than by the blood-agar plate method into α and β types which correspond to Streptococcus viridans and Streptococcus pathogenes (Schottmüller) respectively. They used veal-infusion broth with an initial acidity of 1.0+ and titrated the amount of acid formed with phenolphthalein as an indicator. A final acidity greater than 2.0+ was apparently considered as indicative of fermentation. The carbohydrates selected were dextrose, lactose, maltose, mannite, raffinose, inulin, saccharose, and salicin. Coagulation of milk always coincided with lactose fermentation and their results were constant. Milk and human strains of hemolytic streptococci were differentiated successfully by pathogenicity tests on rabbits and by agglutination. No cross agglutination between α and β types occurred. They point out that the recording of minute cultural characteristics

and of fermentation reactions are particularly valuable in tracing individual strains in experimental and epidemiological work, with the inference that they are not of especial value as a basis for classification.

Hopkins and Lang in a study of one hundred and five strains of streptococci largely from human pathological sources have offered a classification which for clinical purposes is fairly satisfactory. After careful comparative tests they concluded that fermentation tests were of definite value in differentiating pathogenic and saprophytic streptococci from human sources; that the fermentation tests are best performed in meat-infusion litmus broth containing two per cent or more of peptone and one per cent of the fermentable substance, the initial acidity being between 0.0 and 0.5+ to phenolphthalein; that lactose, salicin, raffinose, mannite, and inulin are of differential value; that titration of the amount of acid formed is of no value in classification; that type of chain-formation, the milk-coagulation test, and the neutral-red reaction are not reliable as a means of differentiation; and that tests for ability to hemolyze human blood are of value in that highly-pathogenic strains are usually strongly hemolytic. Similar studies have convinced me that their conclusions in so far as the fermentation reactions are concerned are thoroughly sound. Their somewhat unsatisfactory results with the blood-agar plate method and their failure to use it as the method of choice for primary differentiation have been discussed above. Their classification is given in Table II.:

TABLE II.

Hopkins and Lang's types of streptococci.

	Lactose.	Saccharose.	Salicin.	Raffinose.	Mannite.	Inulin.	Corresponding to Andrewes and Horder's Types.
Pathogenic type	+	+	+	—	—	—	Str. pyogenes.
Saprophytic types:							
a. Salivary:							
1	+	+	—	+	—	—	Str. salivarius.
2	+	+	+	+	—	—	" "
3	+	+	—	+	—	+	" "
4	+	+	—	—	—	—	" mitis.
b. Fecal	+	+	+	—	+	—	" fecalis.

The main point of weakness in this classification is in the failure to recognize that the fermentation of lactose, saccharose, and salicin is characteristic of a certain number of saprophytic streptococci and that the only means of separating the pathogenic and saprophytic streptococci of this fermentation type is by means of the blood-agar method. For example, their strains Nos. 64, 93, 85, 124, 126, 23, and 109 are all classified under the pathogenic type because they ferment lactose, saccharose, and salicin. None of these strains, however, produced hemolysis, and for that reason should more properly be classified as saprophytic organisms of the viridans type.

The most recent contribution to the subject is that of Holman who has based his proposed classification upon a combination of the blood-agar and the cabohydrate fermentation methods. He has made a very careful comparative study of the various methods and variations in technic advocated by previous workers and has come to the following conclusions: (1) The blood-agar plate method is reliable and essential for primary differentiation into two groups, (a) hemolytic, (b) non-hemolytic or green-producing.

(2) The fermentation reactions are of value for further subdivision and must be carried out in a medium which is especially favorable for the growth of streptococci — for this he advocates serum broth. (3) Five days' incubation is necessary before final fermentation results are recorded. (4) The titration method is not of special value for purposes of classification and is too cumbersome and time-consuming for routine work. (5) Andrade's decolorized acid fuchsin is superior to litmus and entirely satisfactory as an indicator of fermentation. (6) Lactose, mannite, salicin, and inulin are the test substances of greatest differential value. Holman's classification is excellent in that it recognizes the value of dividing the streptococci into two large groups — hemolytic and non-hemolytic, or methemoglobin-producing. He has subdivided each of these large groups by means of the fermentation reactions in lactose, salicin, and mannite into eight sub-groups and in addition has divided the sub-groups into inulin and non-inulin fermenters. Such extensive subdivision has resulted in a multiplicity of types which seem to me to be of limited clinical value for reasons which will be presented. Holman's careful comparative study of methods is particularly valuable and the technic and media recommended by him would seem to be thoroughly satisfactory in most respects. The addition of serum to the broth used in the fermentation reactions is perhaps an unnecessary refinement. I have found two per cent peptone meat infusion broth with an initial acidity of 0.0 to 0.5+ thoroughly satisfactory.

In summarizing the work reported by various investigators who have used the carbohydrate fermentation tests as a means of classifying the streptococci, it may be safely said that the continued use of the method in spite of more or less variable results indicates that it is not without considerable value. As has been pointed out, many of the inconsistencies that have appeared are due to failure to use the blood-agar plate method for primary differentiation before the fermentation tests were applied. The use of different media and of various indicators has further complicated the results. That especially favorable media for the growth of streptococci is

essential has developed through the studies of Broadhurst and Holman, and it is quite probable that the use of unfavorable media in some instances has contributed to the difficulties met in the use of the method. The most commonly used sugar media have been litmus serum water, meat-extract broth, meat-infusion broth, and serum broth, to which one per cent of the test carbohydrates have been added. With the broth media, litmus, Andrade's decolorized acid fuchsin, and titration, with phenolphthalein as an indicator, have been used to determine acid production. Comparative studies have shown that broth media are superior to litmus serum water in several respects — briefly, growth of streptococci in suitable broth media is often better than in serum water, the initial acidity of broth media is more easily determined, broth media is more easily prepared, and the results with it have been more consistent than with litmus serum water media. Broadhurst has shown that meat-extract broth is not satisfactory, and it is now generally recognized that meat-infusion broth is preferable. Holman considers that this is not sufficiently favorable for the growth of streptococci, and has advocated the use of serum broth. On the other hand, Hopkins and Lang found a two per cent peptone meat-infusion broth entirely suitable, and consider the addition of serum unnecessary. Holman, Hopkins and Lang, and Hartzell and Henrici, after careful comparative studies, have come to the conclusion that the use of the titration method is cumbersome and of little value for purposes of routine classification, and have found Andrade's decolorized acid fuchsin or litmus thoroughly reliable as an indicator. Similar studies have convinced me that this is true, provided the initial acidity of the broth media is between 0.0 and 0.5 + to phenolphthalein, as recommended by Hopkins and Lang. Sixty fermentation tests were carried out in two per cent peptone meat infusion, one per cent sugar broths, with an initial acidity of 0.3 + to phenolphthalein, each culture tube containing seven to eight cubic centimeters of media. After five days' incubation five cubic centimeters were removed from each tube by pipette and the acidity determined by

titration with phenolphthalein as an indicator: 1.2 + acidity or greater increase above the initial acidity was considered as indicating fermentation. To the remaining two or three cubic centimeters of culture media in each tube litmus was added. Positive and negative results corresponded without exception. With Andrade's indicator I have had no experience, but Holman's work has shown that it is thoroughly satisfactory.

Two further points in the preparation of the media deserve brief mention. Broth sterilized in flowing steam on three successive days is more favorable for growth than broth sterilized in the autoclave, and the former method should be used. The use of broth rendered sugar-free by preliminary incubation with B. coli is undesirable, as such broth is less favorable for growth of streptococci. Holman and others have shown that the amount of acid produced in broth not rendered sugar-free is never sufficient to affect the indicator or to interfere with the results, and I am able to confirm their observations by similar studies.

The duration of incubation before recording results has varied from two to seven days with different observers. Five days' incubation is undoubtedly a safe outside limit if attention is paid to the two following important points in technic which seem to have received little notice from most observers — namely, the method of inoculation of the test-sugar media from the parent culture and the age of the parent culture at the time of inoculation. Gillespie has shown that in inoculating fluid media it is essential that a considerable amount of the parent culture be transferred for growth consistently to occur in the sub-culture, and the recent studies of Chesney on bacterial lag emphasize the importance of using a young actively-growing parent culture for seeding of the sugar media. For satisfactory results it is desirable that inoculations of the sugar media be made from twelve to eighteen-hour parent cultures in broth, .1 to .2 cubic centimeter of the parent culture being transferred by pipette to each tube of the sugar media.

It has been contended by some investigators that the carbohydrate fermentation tests are valueless because they

have shown that many strains of streptococci after cultivation on artificial media for varying periods of time spontaneously gain or lose the power to ferment certain carbohydrates, and that such changes in fermentation reactions may be artificially brought about by subjecting streptococci to special environmental conditions. Few of these observations are convincing and other investigators have been unable to confirm these results, but have found the streptococci remarkably constant in their fermentation reactions. Holman has ably discussed errors which may occur in such studies. It is not the purpose of this paper to enter into a discussion of bacterial mutation. It is only necessary to point out that such changes, even if they do occasionally occur, in no way invalidate the use of the carbohydrate fermentation method as a practical means of identification of the streptococci for clinical purposes, for we are dealing with streptococci as isolated and not after they have been subjected to various artificial conditions.

It is obvious that failure in many instances to appreciate the points in method and technic discussed above has been the source of much of the confusion that has occurred in previously reported work. Careful attention to these points will undoubtedly obviate many of the difficulties previously encountered and it is to be hoped that a definite and consistent method in the use of the carbohydrate fermentation tests will eventually be established. A reduction of the number of test substances to a minimum should tend further to clarify the results and more sharply differentiate the groups.

Classification. — The classification of streptococci to be of value for routine clinical purposes should involve the use of as simple a technic as possible and at the same time should give information as to the predominant types of streptococci associated with a disease process and some indication of the source of the types encountered. The limitation of differential media to a minimum and the avoidance of a cumbersome classification with a multiplicity of types that are of

little practical value is the end to be sought. It is with this purpose in view that the following classification is proposed. In brief the classification depends upon a primary differentiation by means of the blood-agar plate method into two groups: (1) *Streptococcus hemolysans* or *Streptococcus hemolyticus* (see page 121 for reasons for preference for hemolysans) characterized by the production of a true homolysin; (2) *Streptococcus viridans* characterized by the ability to transform oxyhemoglobin to methemoglobin. This group includes also those strains which fail to cause any change in blood. Group 2 is further divided into three groups by the use of lactose and mannite fermentation tests — (a) *Streptococcus buccalis* characterized by the fermentation of lactose and non-fermentation of mannite; (b) *Streptococcus fecalis* characterized by the fermentation of both lactose and mannite; and (c) *Streptococcus equinus* characterized by failure to ferment either lactose or mannite. Table III. presents the classification in tabular form.

TABLE III.

Streptococci.

Gram-positive, bile insoluble cocci in chains

Hemolysis ——— Blood agar ——— Methemoglobin and Indifferent

Hemolysis		Methemoglobin and Indifferent	
		Str. viridans	
Str. hemolysans	Lactose + Mannite —	Lactose + Mannite +	Lactose — Mannite —
	Str. buccalis	Str. fecalis	Str. equinus

The reasons for using the blood-agar plate method for division of the streptococci into two large groups have been stated in part and need only brief repetition. Suffice it to say that the division depends upon characteristic and distinct metabolic functions and that it is of considerable clinical value, at least from the point of view of prognosis, Streptococcus hemolysans being usually parasitic and highly pathogenic, while Streptococcus viridans is either saphrophytic or

if associated with a pathological process is usually of relatively low virulence. While it is realized that a classification dependent upon the antigenic properties of bacteria, such as has been developed for the colon-typhoid, dysentery, and pneumococcus groups is preferable to one dependent upon metabolic functions, this has seemed impossible in the present state of our knowledge of streptococci. Evidence is not lacking, however, that the action of streptococci on blood bears some relation to their antigenic properties, a fact which lends support to the value of primary differentiation into hemolytic and methemoglobin-producing groups.

Ruediger from his studies on agglutinins found that cross-agglutination occurred between strains of hemolytic streptococci, but that hemolytic streptococci were not agglutinated by the serum of an animal immunized to Streptococcus viridans. Davis, from a study of anaphylaxis in cross-sensitization experiments, found a close relationship between hemolytic strains which did not exist between hemolytic and viridans strains. Smith and Brown found no cross-agglutination between their α and β types, though cross-agglutination took place within the individual groups. Kligler's results were somewhat at variance with these as he found a closer relationship in agglutination experiments between strains fermenting the same carbohydrates than between strains having similar hemolytic properties.

A series of agglutination experiments by the author, though somewhat limited in number, lends weight to the value of differentiation by the blood-agar plate method. Twelve immune sera produced by the immunization of rabbits with twelve different strains of Streptococcus viridans, while showing numerous cross-agglutination reactions when tested against forty strains of Streptococcus viridans, failed in any instance to agglutinate six strains of Streptococcus hemolysans. In a similar manner an immune serum produced by the immunization of a rabbit to one of the six strains of hemolytic streptococci showed cross-agglutination with three of the six hemolytic strains, but failed to agglutinate any of the forty viridans strains.

Methods. — The following methods have been used in the work reported below and have proved satisfactory:

Media: The media used have been practically the same throughout and were prepared as follows:

Blood agar: Plain agar (0.3+ to 0.6+) in tubes containing about fifteen cubic centimeters each was melted, cooled to about 45° C., and 1.5 cubic centimeters of sterile defibrinated human or rabbit's blood were added by pipette. The tube was then inoculated, an even mixture of the blood and agar being obtained by pouring into a sterile test-tube and back before plating. If this is carefully done the danger of contamination is negligible and the value of an even mixture of the blood and agar far outweighs the slight chance of contamination. By this method colonies are well separated and easy to fish. What is more important, a much better idea of the relative proportion of various organisms present in the material inoculated can be obtained than often is possible by the use of the surface-streak method.

Carbohydrate media: Two per cent peptone meat-infusion broth, 0.0–0.5+ acidity, containing one per cent of the test-carbohydrate and sterilized in flowing steam on three successive days has been used throughout. Media was incubated for forty-eight hours before being stored on ice to make sure that no contamination was present. Seven to eight cubic centimeters of culture media was used in each culture-tube. Lactose, mannite, salicin, saccharose, raffinose, and inulin were used as test substances. In the classification proposed only lactose and mannite were retained as being of differential value.

Isolation: In the case of material in which more than one type of bacterium was presumably present, such as throat cultures, the material was inoculated in broth and sub-cultures immediately made in blood-agar plates, thus serving to give an idea of the relative proportion of different types of organisms in the original material. After twenty-four hours' incubation colonies appearing to be hemolytic and methemoglobin-producing streptococci were fished,

smears made for microscopic examination, Gram and capsule strains, and sub-cultures made in dextrose broth and on ascitic-agar slants for storage. If any reasonable doubt existed as to the purity of the colonies fished, replating in blood agar was resorted to.

Reaction on blood: The presence of hemolysis or of methemoglobin formation on blood-agar plates was recorded after twenty-four hours' incubation. In addition about one hundred strains were tested by adding one cubic centimeter of eighteen-hour ascitic-broth cultures to one cubic centimeter of a five per cent suspension of washed sheep corpuscles in small test-tubes and incubating for two hours at 37° C.

Growth in broth: Characteristics of the growth in broth were recorded after twenty-four hours' incubation, dextrose broth being the media used.

Chain formation: The length of chains was noted after twenty-four hours' growth in dextrose broth. Because of the variation in chain length which may occur in a single culture such observations are of doubtful value. The predominant type of chain formation was the one recorded as characteristic of the strain. At the same time observations were made as to morphology.

Bile solubility: All non-hemolytic strains were tested for bile solubility by the addition of one or two drops of rabbit's bile to .5 cubic centimeter of a salt solution suspension of the organism. No bile soluble strains are included in the present study.

Fermentation: The fermentation tests were carried out as soon as possible after isolation. One-tenth to two-tenths cubic centimeter of a twelve to eighteen hours broth culture was added by pipette to each of the sugar media. After five days incubation the presence or absence of fermentation was recorded. In the present work the acid formed was determined by titration with phenolphthalein as an indicator, five cubic centimeters of the culture media being removed from the culture tube by pipette for titration. An increase in acidity of 1.2+ or more above the initial acidity of the

media was considered as indicative of fermentation. In a considerable series, as stated above, litmus was added to the remaining media in the tube, parallel results being obtained throughout. Further use of the titration method for purposes of classification seems unnecessary.

Streptococcus hemolysans or Streptococcus hemolyticus. — The majority of classifications presented have subdivided the hemolytic streptococci by means of the fermentation reactions, but I have found little practical advantage in such subdivision. The majority of hemolytic streptococci conform to the type fermenting lactose, saccharose, and salicin. Variants from this type occur in which one or two of these substances are not fermented or in which mannite or, rarely, raffinose is fermented in addition. In analyzing the work of others and in my own work I have not found that there is any relationship between these various fermentation types and the source, immunological reactions or pathogenicity of the strains encountered, and therefore do not consider subdivision of practical value.

Holman has subdivided the hemolytic streptococci into eight groups. Four of these contain but very few strains and are of minor importance. The remaining four groups each contain a considerable number of strains, but if the sources from which these strains are derived are tabulated, it is found that they are identical and in relatively the same percentage for each of the four groups. For example, if the strains isolated from the blood in cases of septicemia are considered, it is found that they are composed of 10.6 per cent of the total number of strains of Streptococcus infrequens, 10.7 per cent of the total number of strains of Streptococcus pyogenes, 11.4 per cent of the total number of strains of Streptococcus anginosus, and 9.1 per cent of the total number of strains of Streptococcus subacidus. The same comparison holds roughly true for strains from other disease sources. Ruediger divided the hemolytic streptococci into two groups, the one fermenting mannite, the other not, but he failed to advance any practical reason for so doing. In

fact, agglutination experiments carried out by him showed no relationship between the fermentative characteristics and antigenic properties of the strains studied, cross-agglutination occurring among mannite and non-mannite fermenters. Andrewes and Horder, Broadhurst and others, have recognized two types of hemolytic streptococci dependent upon fermentation reactions, Streptococcus pyogenes and Streptococcus anginosus. They have, however, demonstrated no other individual characteristics which warrant such a division. Henrici has recently shown that there is no relationship between fermentation types and virulence, and considers it doubtful whether carbohydrate fermentation tests are of significance from the standpoint of tissue localization.

For these reasons it seems to me to be of little practical value to subdivide the hemolytic streptococci by means of fermentation reactions, but much more logical to group them together. I have used the name Streptococcus hemolysans for this group of streptococci because it denotes the characteristic metabolic function of the group, and because it is comparable to the accepted name of the methemoglobin-producing group, Streptococcus viridans. It furthermore obviates the confusion that might arise from the use of the name Streptococcus pyogenes, which has been variously used by different investigators. Streptococcus hemolyticus is the name used previously in much the same sense as I have used Streptococcus hemolysans, and possibly this term should be retained on account of its very general use, although the term Streptococcus hemolysans seems preferable.

In Table IV. are shown the fermentation reactions, source, characteristics of the growth in bouillon, and type of chain formation of fifty-one strains of Streptococcus hemolysans studied by the author.

TABLE IV.

Streptococcus hemolysans.

Streptococcus.		Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
AA	7.2 . .	Alveolar abscess	+	—	+	+	—	—	S	M
C	1 B . .	Blood, sepsis	+	—	+	+	—	—	S	L
C	1 J . .	Elbow joint, sepsis	+	—	+	+	—	—	S	L
I	J . . .	Pus, abscess	+	—	+	+	—	—	S	L
M	1 . . .	Spinal fluid, meningitis	+	—	+	+	—	—	S-D	L
P	6 . . .	Sputum, pneumonia	+	—	+	+	—	—	S	L
P	13 . . .	Blood, sepsis	+	—	+	+	—	—	S	L
R	S 1 . .	Rat, sepsis	+	—	+	+	—	—	S	L
S	2 . . .	Blood, scarlet fever	+	—	+	+	—	—	S	L
S	F 3 . .	Joint, scarlet fever	+	—	+	+	—	—	S	L
S	T 1 . .	Pus, tendon sheath	+	—	+	+	—	—	S	M
S	T 2 . .	" " "	+	—	+	+	—	—	S	M
T	12 a . .	Tonsil, acute tonsillitis	+	—	+	+	—	—	S-D	L
T	20.3 . .	" " "	+	—	+	+	—	—	S	L
T	28.1 . .	" " "	+	—	+	+	—	—	S	M
T	29.1 . .	" " "	+	—	+	+	—	—	S	L
T	30 . .	" " "	+	—	+	+	—	—	S	L
T	31.1 . .	" " "	+	—	+	+	—	—	S	L
T	32 . .	" " "	+	—	+	+	—	—	S	L
T	35.1 . .	" " "	+	—	+	+	—	—	S	M
T	36.1 . .	" " "	+	—	+	+	—	—	S	L
T	37 . .	" " "	+	—	+	+	—	—	S	L
T	37.E .	" " "	+	—	+	+	—	—	S-D	L
T	38 . .	" chronic "	+	—	+	+	—	—	S	M
T	39.1 . .	" acute "	+	—	+	+	—	—	S	L
T	41.1 . .	" " "	+	—	+	+	—	—	S	L
T	42.1 . .	" " "	+	—	+	+	—	—	D-S	L
T	43.1 . .	" " "	+	—	+	+	—	—	S	L
T	44.1 . .	" " "	+	—	+	+	—	—	S-D	S
T	45.1 . .	Throat, acute pharyngitis	+	—	+	+	—	—	S	L
T	46.1 . .	Tonsil, chronic tonsillitis	+	—	+	+	—	—	S	L
T	47.1 . .	Throat, acute pharyngitis	+	—	+	+	—	—	D	S

TABLE IV. — *Continued.*

Streptococcus.		Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
T	49 . .	Tonsil, acute tonsillitis	+	—	+	+	—	—	S	L
T	50.1 . .	" " "	+	—	+	+	—	—	S	L
T	53.1 . .	" chronic "	+	—	+	+	—	—	S	L
T	54.L.1 .	" " "	+	—	+	+	—	—	S	L
T	54.2 . .	" " "	+	—	+	+	—	—	S	L
T	54.3 . .	" " "	+	—	+	+	—	—	S	L
T	25.1 . .	" " "	+	+	+	+	—	—	S-D	L
T	25.2 . .	" " "	+	+	+	+	—	—	S-D	L
T	25.3 . .	" " "	+	+	+	+	—	—	S-D	L
T	25.4 . .	" " "	+	+	+	+	—	—	S-D	L
T	33.1 . .	" acute "	+	+	+	+	—	—	S	L
T	48 . .	" " "	+	—	—	+	—	—	S	L
T	51.1 . .	" " "	+	—	—	+	—	—	S	LL
S	K 1 . .	Knee joint, sepsis	—	—	+	+	—	—	S	L
T	23.1 . .	Tonsil, chronic tonsillitis	—	—	+	+	—	—	S-D	L
T	23.2 . .	" " "	—	—	+	+	+	—	S-D	L
T	21.L.1 .	" " "	+	—	+	+	+	—	S	L
T	21.L.2 .	" " "	+	—	+	+	+	—	S	L
T	21.R.1,	" " "	+	—	+	+	+	—	S	M

+ indicates fermentation; — indicates no fermentation. Bouillon: S indicates sedimentation, S-D indicates sedimentation with slight diffuse turbidity, D-S indicates diffuse turbidity with slight sedimentation, D indicates diffuse turbidity. Chain formation: S indicates short chains, M indicates chains of medium length, L indicates long chains, LL indicates chains of extreme length.

Streptococcus viridans. — Subdivision of the methemoglobin-producing group of streptococci by means of fermentation reactions has seemed of some value in that it serves to provide an indication of the source of individual strains, a point of considerable importance in dealing with focal infections and the metastatic lesions arising therefrom. In order to avoid a useless multiplicity of types and to limit the subdivisions to such as are of definite value, but three subgroups have been made, with the use of only lactose and

mannite as differential media. These groups are (1) Streptococcus buccalis, the type commonly found in the mouth; (2) Streptococcus fecalis, the type characteristically found in the gastro-intestinal tract of man; (3) Streptococcus equinus, the type commonly found in horse feces, street dust, etc., and so occasionally encountered in the human nose and throat.

Streptococcus buccalis: The type of streptococcus commonly found in the mouth has been divided into two groups — Streptococcus mitis and Streptococcus salivarius — by most authors, and even further subdivisions have been made by some. These subdivisions into various types have depended solely upon the fermentation reactions, and are of little apparent practical value. Certainly strains of Streptococcus mitis and Streptococcus salivarius do not possess any individual type characteristics which would warrant their separation from a clinical or pathological standpoint. Long and short-chained types, raffinose and non-raffinose fermenters, strains growing as a sediment in broth and those growing diffusely occur impartially in both groups. Their normal habitat is the same, they are associated with identical pathological processes, and no evidence has been produced to show that they possess individual antigenic properties. These conclusions are inevitable after a careful analysis of the work reported by others, and are substantiated by the results shown in Table V.

For the foregoing reasons it has seemed to me advisable to group Streptococcus mitis, Streptococcus salivarius, and such other mouth types as have been described together, and to this group I have applied the name Streptococcus buccalis as indicative of the source from which such streptococci are commonly derived.

The fermentation reactions of different strains of mouth streptococci are variable, and their reactions in the six commonly used differential carbohydrates may be represented as follows: lactose +, saccharose +, salicin ±, raffinose ±, inulin ∓, mannite –. Of these, the reaction in lactose is

consistently positive, in mannite negative. These two reactions alone serve to distinguish Streptococcus buccalis from Streptococcus fecalis and Streptococcus equinus, and in the proposed classification the other carbohydrates have been discarded as of no differential value. In brief, Streptococcus buccalis includes all strains of Streptococcus viridans that ferment lactose and fail to ferment mannite.

In Table V. are presented the source, fermentation reactions, characteristics of the growth in broth, and type of chain formation of ninety-eight strains of Streptococcus buccalis studied by the author.

TABLE V.

Streptococcus buccalis.

Streptococcus.	Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
N 1.3	Urine, acute nephritis	+	—	+	—	—	—	D-S	S
AA 2	Alveolar abscess	+	—	—	+	—	—	S	L
AA 6.2	" "	+	—	—	+	—	—	S	M
AA 6.4	" "	+	—	—	+	—	—	S-D	L
AA 6.5	" "	+	—	—	+	—	—	S	L
AA 9	" "	+	—	—	+	—	—	D	S
E 12 V 3	Heart valve, endocarditis	+	—	—	+	—	—	D-S	M
R 1	Tonsil, rheumatic fever	+	—	—	+	—	—	D-S	M
S H	Mouth, stomatitis	+	—	—	+	—	—	S	LL
T 2	Tonsil, acute tonsillitis	+	—	—	+	—	—	S	L
T 42.3	" " "	+	—	—	+	—	—	D-S	M
T 51.2	" " "	+	—	—	+	—	—	S-D	M
T 52	" chronic "	+	—	—	+	—	—	S	L
T 53.2	" " "	+	—	—	+	—	—	S	L
T 41.2	" acute "	+	—	—	+	—	+	D	L
AA 6.3	Alveolar abscess	+	—	+	+	—	—	D	S
AA 8.1	" "	+	—	+	+	—	—	D	S
AA 8.2	" "	+	—	+	+	—	—	D	S
AA 13	" "	+	—	+	+	—	—	S	M

TABLE V. — *Continued.*

Streptococcus.	Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
E 12 V 2	Heart valve, endocarditis	+	−	+	+	−	−	D-S	S
P 18	Sputum, pneumonia	+	−	+	+	−	−	S-D	M
T 7	Tonsil, acute tonsillitis	+	−	+	+	−	−	S	L
T 9	" " "	+	−	+	+	−	−	S	L
T 19.6	" " "	+	−	+	+	−	−	D	S
T 24 R	" chronic "	+	−	+	+	−	−	S	L
T 26.1	" acute "	+	−	+	+	−	−	S-D	M
T 28.2	" " "	+	−	+	+	−	−	S	L
T 47.2	Throat, acute pharyngitis	+	−	+	+	−	−	D-S	M
T 20.1	Tonsil, acute tonsillitis	+	−	+	+	−	+	S-D	L
AA 3 b 1	Alveolar abscess	+	−	−	+	+	−	D-S	M
AA 3 b 2	" "	+	−	−	+	+	−	D-S	M
AA 3 b 4	" "	+	−	−	+	+	−	D-S	M
AA 3 a	" "	+	−	−	+	+	−	D-S	S
AA 6.6	" "	+	−	−	+	+	−	S-D	L
AA 6.9	" "	+	−	−	+	+	−	S	L
AA 10.2	" "	+	−	−	+	+	−	D-S	S
AA 11.1	" "	+	−	−	+	+	−	D-S	M
AA 11.2	" "	+	−	−	+	+	−	D-S	S
E 12 b	Blood, endocarditis	+	−	−	+	+	−	D-S	S
E 12 V 4	Heart valve, endocarditis	+	−	−	+	+	−	D-S	S
P 3	Sputum, pneumonia	+	−	−	+	+	−	D-S	M
SF 1	Tonsil, scarlet fever	+	−	−	+	+	−	D	S
T 1	" acute tonsillitis	+	−	−	+	+	−	D	M
T 5	" " "	+	−	−	+	+	−	S	M
T 11	" " "	+	−	−	+	+	−	S-D	L
T 12 b	" " "	+	−	−	+	+	−	S	L
T 14	" " "	+	−	−	+	+	−	S	L
T 34	" " "	+	−	−	+	+	−	D	L
T 39.2	" " "	+	−	−	+	+	−	S	L
T 39.4	" " "	+	−	−	+	+	−	S-D	L
T 44.2	" " "	+	−	−	+	+	−	S-D	M
T 50.3	" " "	+	−	−	+	+	−	D-S	S

TABLE V. — *Continued.*

Streptococcus.	Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
AA 3 b 3 .	Alveolar abscess	+	—	—	+	+	—	D-S	L
AA 6.7 . .	" "	+	—	—	+	+	+	S	L
E 16 . . .	Blood, endocarditis	+	—	—	+	+	+	S-D	M
T 22 . . .	Tonsil, acute tonsillitis	+	—	—	+	+	+	D-S	M
AA 5 a . .	Alveolar abscess	+	—	+	+	+	—	D-S	L
AA 5 b . .	" "	+	—	+	+	+	—	D-S	L
AA 6.8 . .	" "	+	—	+	+	+	—	S	L
AA 7.1 . .	" "	+	—	+	+	+	—	S-D	S
AA 10.1 . .	" "	+	—	+	+	+	—	D-S	L
D 1 a . .	Tonsil, diphtheria	+	—	+	+	+	—	D	L
P 10 . . .	Sputum, pneumonia	+	—	+	+	+	—	D-S	L
R 8 v . .	Heart valve, rheumatic endocard.,	+	—	+	+	+	—	D-S	S
T 3 . . .	Tonsil, acute tonsillitis	+	—	+	+	+	—	D	S
T 4 . . .	" " "	+	—	+	+	+	—	S-D	S
T 12 c . .	" " "	+	—	+	+	+	—	D-S	M
T 16 . . .	" " "	+	—	+	+	+	—	D-S	M
T 18 a . .	" " "	+	—	+	+	+	—	D-S	M
T 20.2 . .	" " "	+	—	+	+	+	—	D	L
T 20.4 . .	" " "	+	—	+	+	+	—	D-S	M
T 20.5 . .	" " "	+	—	+	+	+	—	S-D	L
T 26.3 . .	" " "	+	—	+	+	+	—	D	S
T 29.2 . .	" " "	+	—	+	+	+	—	D-S	M
T 42.2 . .	" " "	+	—	+	+	+	—	S-D	L
T 45.2 . .	Throat, acute pharyngitis	+	—	+	+	+	—	S	M
AA 6.1 . .	Alveolar abscess	+	—	+	+	+	+	S-D	S
E 3 . . .	Blood, endocarditis	+	—	+	+	+	+	D-S	S
P 8 . . .	Sputum, pneumonia	+	—	+	+	+	+	D-S	S
T 6 . . .	Tonsil, acute tonsillitis	+	—	+	+	+	+	D-S	L
T 8 . . .	" " "	+	—	+	+	+	+	S	L
T 12 d . .	" " "	+	—	+	+	+	+	D-S	S
T 13 . . .	" " "	+	—	+	+	+	+	D-S	L
T 15 . . .	" " "	+	—	+	+	+	+	D	M
T 17 . . .	" " "	+	—	+	+	+	+	D-S	M

TABLE V. — *Concluded.*

Streptococcus.	Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
T 18 b . .	Tonsil, acute tonsillitis	+	—	+	+	+	+	D-S	M
T 19.1 . .	" " "	+	—	+	+	+	+	D-S	S
T 24 L .	" chronic "	+	—	+	+	+	+	S	L
T 26.2 . .	" acute "	+	—	+	+	+	+	S-D	L
T 27.4 . .	" " "	+	—	+	+	+	+	D	S
T 31.2 . .	" " "	+	—	+	+	+	+	D	S
T 39.3 . .	" " "	+	—	+	+	+	+	D	S
T 40.1 . .	" " "	+	—	+	+	+	+	D	S
T 40.2 . .	" " "	+	—	+	+	+	+	D	S
T 46.2 . .	" chronic "	+	—	+	+	+	+	S	L
T 54 L 2 .	" " "	+	—	+	+	+	+	S	L
T 54.1 . .	" " "	+	—	+	+	+	+	D-S	S
W 10 . . .	Sputum, chronic bronchitis . . .	+	—	+	+	+	+	D	S

+ indicates fermentation; — indicates no fermentation. Bouillon: S indicates sedimentation, S-D indicates sedimentation with slight diffuse turbidity, D-S indicates diffuse turbidity with slight sedimentation, D indicates diffuse turbidity. Chain formation: S indicates short chains, M indicates chains of medium length, L indicates long chains, LL indicates chains of extreme length.

Streptococcus fecalis: It has been thoroughly established that the majority of streptococci from the intestinal tract of man are characterized by the fermentation of mannite. The name Streptococcus fecalis was introduced by Andrewes and Horder for this group and it has found a place in the classifications of practically all authors who have used the fermentation method. That strains of streptococci which fail to ferment mannite do occur in human feces need not invalidate this group, for it is highly probable that such strains have originated in the mouth and they may quite properly be classified as Streptococcus buccalis. Although an immunological basis for the acceptance of the group is entirely wanting, it nevertheless seems of practical value to recognize the group because to do so affords definite information as to the source of the streptococci so classified. Strains of

Streptococcus viridans that ferment lactose and mannite are therefore grouped together in the proposed classification and the name Streptococcus fecalis used for the group.

In Table VI. are presented the source, fermentation reactions, characteristics of the growth in broth, and type of chain formation of strains of Streptococcus fecalis studied by the author.

TABLE VI.

Streptococcus fecalis.

Streptococcus.	Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
AA 1 . . .	Alveolar abscess	+	+	+	+	—	—	D	S
N 1.2 . .	Urine, acute nephritis	+	+	+	+	—	—	D	S
T 11.a . .	Abscess, pyemia	+	+	+	+	+	—	D-S	S
N 1.4 . .	Urine, acute nephritis	+	+	+	+	+	—	D	S
T 33.2 . .	Tonsil, acute tonsillitis	+	+	+	+	+	+	S-D	M
N 1.1 . .	Urine, acute nephritis	+	+	+	+	+	+	D-S	S
N 1.5 . .	" " "	+	+	+	+	+	+	D-S	S
T 50.2 . .	Tonsil, acute tonsillitis	+	+	+	+	+	+	S-D	L

Streptococcus equinus: Gordon, and Andrewes and Horder showed that strains of streptococci from horse feces and street dust are characterized by failure to ferment lactose, and their findings have been thoroughly substantiated by other observers. To this group of streptococci the name Streptococcus equinus has been applied. While strains of Streptococcus viridans, which fail to ferment lactose are not commonly found in normal or disease sources in man, they are, nevertheless, occasionally encountered, and therefore it has seemed advisable to recognize the group for the same reasons that have been stated with respect to Streptococcus buccalis and Streptococcus fecalis. In the proposed classification, then, strains of Streptococcus viridans that fail to ferment lactose and mannite are grouped together and called Streptococcus equinus.

In Table VII. are presented the source, fermentation reactions, characteristics of the growth in broth, and type of chain formation of strains of Streptococcus equinus studied by the author.

TABLE VII.

Streptococcus equinus.

Streptococcus.	Source.	Lactose.	Mannite.	Salicin.	Saccharose.	Raffinose.	Inulin.	Bouillon.	Chain Formation.
D 1 c	Tonsil, diphtheria	—	—	—	+	—	—	S	L
E 12 v 1	Heart, valve endocarditis	—	—	+	+	+	+	D	M
S F 2	Tonsil, scarlet fever	—	—	+	+	+	+	D-S	M
T 10	Tonsil, acute tonsillitis	—	—	+	+	+	+	D-S	M

Unclassified: In the above classification no provision has been made for strains of Streptococcus viridans that ferment mannite and do not ferment lactose. Such strains, however, are extremely rare. I have encountered but one and have found only four others reported in the literature, three of which were from the intestinal tract of guinea-pigs. It is possible that such strains should be included in the fecalis group. Certainly they are not of sufficient number or importance to warrant a separate group, and in order that the proposed grouping may be clear cut I have preferred to allow these few strains to remain unclassified.

SUMMARY.

The classification of the streptococci is at present in a state of confusion. This confusion has largely been due to failure to recognize that a primary division of streptococci by the blood-agar plate method is essential, and to a wide variation in the methods, technic, and media used by various investigators. A critical analysis of previously reported work has served in some degree to show in detail the

reasons for the inconsistencies in classification that have occurred.

A practical and simple method of classification of the streptococci for clinical purposes is desirable. While it is recognized that the ultimate goal to be attained is a classification founded upon an immunological basis, such a classification is at present unavailable. The classification proposed is dependent upon a combination of the blood agar and carbohydrate fermentation tests, lactose and mannite only being used for the latter. A standard technic should be adopted and the following, which has been discussed in detail above, is recommended. In the blood-agar plate method five to ten per cent of defibrinated blood should be used, the blood and agar should be evenly mixed, the colonies should be well separated, and the results should be recorded at the end of twenty-four hours' incubation. In the fermentation method two per cent peptone meat-infusion broth containing one per cent of the test-carbohydrate, initial acidity 0.0 to 0.5+ to phenolphthalein, sterilized by the fractional method in flowing steam should be used. Lactose and mannite are of differential value and further tests are unnecessary. Litmus or Andrade's decolorized acid fuchsin should be used as an indicator, titration of the acidity produced being of no additional value. Inoculation of the sugar media should be made from actively-growing parent cultures, .1 to .2 cubic centimeter being used for the seeding of each tube. Five days' incubation should be allowed before recording final results. By this method a classification of the streptococci of distinct clinical value may be made and is as follows: (1) Streptococcus hemolysans, characterized on blood-agar plates by colonies surrounded by a wide zone of hemolysis; (2) Streptococcus viridans, characterized by the formation of methemoglobin or failure to alter blood and distinguishable from the pneumococcus by being bile insoluble. Further subdivision of Streptococcus hemolysans by means of fermentation reaction is of no practical value. Streptococcus viridans, however, is divided into three sub-groups by its fermentation reactions in lactose and mannite, a procedure which is of

value in that it gives some indication of the source of the strain in question. These sub-groups are (a) Streptococcus buccalis characterized by the fermentation of lactose and failure to ferment mannite, (b) Streptococcus fecalis characterized by the fermentation of both lactose and mannite, and (c) Streptococcus equinus characterized by the failure to ferment either lactose or mannite. Limitation of the carbohydrate-fermentation reactions to the use of lactose and mannite reduces the number of unclassified or variant strains to a negligible minimum.

While it is fully recognized that more extensive study of the growth-characteristics, pathogenicity, and metabolic activities of various strains of streptococci are of much value in experimental and epidemiological work, such investigations are too cumbersome and time consuming, and of insufficient value for purposes of routine identification. It is believed that adoption of the proposed method of classification will serve to obviate the confusion that has hitherto existed and to provide a nadequate means of identification of streptococci for clinical purposes until such time as an immunological basis for classification shall be developed.

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CULTURAL AND ANTIGENIC DIFFERENCES IN STRAINS OF BACILLUS TYPHOSUS AND STUDIES IN THE PARA-TYPHOID GROUP.*

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GENERAL. — Although no other class of microörganisms has received more attention from bacteriologists than the colon, typhoid, paratyphoid, and dysentery groups, our knowledge concerning them is, to a large extent; unsatisfactory. Especially is this true of the paratyphoid organisms and other members of the so-called "intermediate" group. It has remained a task requiring much care and labor to identify any unknown strain which by the simpler cultural tests was found to conform with the general characteristics of the "paratyphoid" or "intermediate" group, and when the knowledge of the source of such an organism was unavailable, it has (with the single exception of B. paratyphosus α) been impossible to absolutely identify it.

Although it has been possible to establish two groups of paratyphoid bacilli from human sources — the α and the β — yet even to do this it has been necessary to resort to agglutination tests for a positive identification, since cultural differences between them were not as constant and decisive in all cases as would seem desirable. Moreover, within the α and β groups, members have been found which were more or less atypical, and organisms have been described which, though obviously belonging to this "intermediate" class have not conformed strictly to the established groups. Many bacilli isolated from domestic animals fall into this class, and it has never been clear whether or not these — usually named according to the animal source from which they were derived — were to be considered as separate groups, or whether they were identical with those occurring in man. This aspect,

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therefore, has had sanitary as well as purely bacteriological interest.

Stimulated to some extent by the recent successful division of the pneumococci into antigenic sub-groups, and also by the observation that in the routine examination of typhoid cultures differences were frequently noted, we added to this work a study of typhoid organisms. It seemed that typhoid bacilli also might differ from one another sufficiently to warrant a division of the group, and, indeed, this has been the result of the work.

No classification of strains of B. typhosus and of the subgroups of B. paratyphosus and its allies has to my knowledge been published. (Since the completion of this work an article has appeared by Sanford B. Hooker in The Journal of Immunology of December, 1916, which shows that strains of B. typhosus show antigenic differences, and upon which basis a grouping has been offered.) It is studies upon these groups which form the subject of this paper, and they attempt to show that such a classification exists.

The cultural reactions were studied on thirteen differential media, upon eight of which complete results can at present be reported. These are dextrose, mannite, maltose, and xylose, lactose, saccharose, rhamnose, and inosite. The others we hope to submit in supplementary papers. In the antigenic investigations both agglutination and adsorption reactions were used. In every case where an atypical reaction occurred the test was repeated, and not accepted unless checked, and the possibility of contamination eliminated by plating out the culture.

We have collected strains from many other laboratories, and have isolated a considerable number ourselves.

The cultures studied were:

B. typhosus	31	strains.
B. paratyphosus α	20	"
B. paratyphosus β	25	"
B. choleræ sius	7	"
B. typhi murium	5	"
B. abortus	2	"
Heterogeneous paratyphoid	21	"
Total	111	"

SECTION I.

B. TYPHOSUS. — The fact that all strains of B. typhosus did not conform to one single type was early recognized, but no classification of the differences or any sub-grouping based upon them has yet been published.

Thus Pfeiffer and Kolle[1] observed that various strains of typhoid bacilli behaved differently in vivo. Durham[2,3] found slight differences in the reaction of typhoid bacilli in B. enteritidis Gärtner serum, Friedberger,[4] Kutscher and Meinicke,[5] and others also found race differences. The last-named authors tried to offer an explanation for the antigenic differences observed by the assumption that the "Receptorenapparat" for all the named bacteria may be alike, but that the avidity causes fluctuations among organisms which have like receptors. Cole[6] found considerable variation in the agglutination of five typhoid strains upon typhoid serum. He also obtained different degrees of adsorption of a typhoid serum with three typhoid antigens.

In the investigations of B. typhosus, we studied the properties of thirty-one strains coming from a variety of different sources. Five are old laboratory cultures which have been growing upon artificial media for a number of years. One is the B. typhi abdominalis Rawlings strain which we obtained from the New York City Board of Health. Another was isolated from the spinal fluid of a case of typhoid meningitis which had come to autopsy. The remaining twenty-four strains are of comparatively recent isolation from the blood and feces of typhoid fever patients.

Cultural reactions. — The carbohydrate media used consisted of a sugar-free meat infusion base containing one per cent peptone and .5 per cent salt. To this was added one per cent of the carbohydrate, and the media then sterilized by the fractional method. Care was taken to obtain an absolutely sugar-free base, and to prevent the breaking down of the added carbohydrate by heat.

Dextrose: In dextrose broth thirty strains reacted typically, producing acid in twenty-four hours. One strain, No. 31 K, fermented the sugar slowly.

Mannite: In mannite broth twenty-nine strains gave typical reactions. These produced acid in twenty-four hours. Two strains, one called Plosser, the other Novy, failed to ferment until the third day. They differ from one another culturally on litmus milk, the Plosser type acting like paratyphoid β or "blue typhoid," while the Novy type gives a slight acid reaction, which persists after twenty-three days' incubation. They also differ antigenically, as our later findings showed.

Maltose: The maltose fermentations parallelled those found in the case of dextrose. Thirty strains produced acid in twenty-four hours, while the atypical strain No. 31 K fermented only after a prolonged incubation of three days.

Lactose: In lactose broth all the strains were uniformly negative.

Saccharose: In saccharose broth all the strains were uniformly negative.

Xylose: The most marked cultural variations in the typhoid group occurred in the xylose fermentations. Of the thirty-one strains, twenty-two produced acid in twenty-four hours, three acted slowly, fermenting sufficiently for a positive reading on the third day, and six remained negative after a prolonged incubation. One of the latter is the Rawlings strain. Four of them are atypical antigenically, failing to adsorb out typhoid agglutinins.

Rhamnose: The thirty-one strains of B. typhosus did not ferment rhamnose. As shown by Uhlenhüth this non-fermentation of rhamnose differentiates B. typhosus from all the classified members of the paratyphoid-enteritidis group.

Inosite: Inosite is a cyclohexanhexol and not a carbohydrate. We found that none of our typhoid strains fermented it. (Mr. J. L. Rice of the Roosevelt Hospital Laboratory was my co-worker on inosite fermentation.)

A grouping of these thirty-one strains on a basis of their cultural reactions here given divides them into three distinct types. A diagramatic arrangement of these types is shown in Table I.:

TABLE I.

Cultural variations in strains of Bacillus typhosus.

Type.	Strains.	Dextrose.	Mannite.	Maltose.	Lactose.	Saccharose.	Xylose.	Rhamnose.	Inosite.	Litmus Milk.
1.	B. typhosus Z.	Acid, 24 hours.	Acid, 24 hours.	Acid, 24 hours.	Negative.	Negative.	Acid, 24 hours.	Negative.	Negative.	
	" G. Norris.									
	" Bull.									
	" R.I.									
	" P. & S.									
	" Han. W.									
	" No. 12 W.									
	" Geary.									
	" 28 K.									
	" 34 K.									
	" 36 K.									
	" No. I W.P.									
	" Ruby.									
	" Schaffer.									
	" Moore s.f.									
	" Hint.									
	" Moore bl.									
	" Ryan.									
	" Roth.									
	" Iverson.									
	" 21 K.						Acid, 2–3 days.			
	" 27 K.									
	" Novy.		Acid, 3 days.				Acid, 24 hrs.			Slight acid, 2–3 days.
	" 31 K.	Acid, 2 days.	Acid, 24 hrs.	Acid, 3 days.			Acid, 2 days.			
2.	B. typhosus Rawlings.	Acid, 24 hours.	Acid, 24 hours.	Acid, 24 hours.	Negative.	Negative.	Negative.	Negative.	Negative.	
	" No. 2 W.P.									
	" Dohaney.									
	" Cornwell.									
	" Felix.									
	" Army.									
3.	B. typhosus Plosser.	Acid, 24 hrs.	Acid, 3 days.	Acid, 24 hrs.	Neg.	Neg.	Acid, 24 hrs.	Neg.	Neg.	Initial acidity; alkaline after five days.

The first and apparently most common type is represented in our grouping by twenty-four strains. Twenty of these are culturally typical in all respects, producing acid in dextrose, mannite, maltose, and xylose in twenty-four hours, and giving a negative fermentation on lactose, saccharose, rhamnose, and inosite. The other four are atypical in the rapidity of fermentation of one or more of the carbohydrates. Thus, Nos. 21 K and 27 K produce acid on xylose only after two to three days' incubation. B. typhosus Novy ferments mannite sufficiently for an acid reaction after three days. B. typhosus No. 31 K gave a slow fermentation of dextrose, maltose, and xylose, producing acid only after two to three days' incubation.

The second type, represented by six strains, is uniformly negative on xylose. This differentiates it sharply from the first group, which produces acid on xylose.

The third type, represented by the single strain B. typhi abdominalis Plosser, a non-motile, non-agglutinating organism, is typical culturally in all respects with the exceptions that it ferments mannite slowly, producing acid only after three days, and gives a paratyphoid B. or "blue typhoid" reaction on litmus milk, beginning with faint acidity and turning to sharp alkalinity on the fifth day. (This type differs from B. sanguinarius in its absolute lack of agglutinating or antigenic properties.)

The cultural reactions are still to be amplified, but as far as they go they show that very marked cultural differences do occur. Much further study with a larger number of organisms, upon a greater variety of media, may permit a final grouping.

Antigenic studies. — Earlier in this paper we spoke of the differences observed by Pfeiffer and Kolle, Durham, Friedberger, Kutcher and Meinicke, and others between races of B. typhosus. Their observations were the result of antigenic studies, and the variations they speak of are, therefore, antigenic differences. We have been able to confirm their findings, and to further show that these differences divide the

B. typhosus group into several sub-groups. We have also attempted to parallel this antigenic sub-grouping with that already made on the basis of biological activity.

In all our experiments rabbits were used. Four different typhoid sera were made. Upon three of these nineteen of the strains were agglutinated, and observations were made upon the character of the reaction and the final dilution in which it occurred. Adsorptions were made, using each of the sera with every strain. At the same time the group reaction between typhoid and paratyphoid α and β, and also B. typhi murium was studied agglutinatively. In this part of the investigation two paratyphoid α sera, three paratyphoid β sera, and one B. typhi murium serum were used.

Agglutination reactions. — All serum dilutions and antigen emulsions were made with isotonic salt solution, and the tests set up with one-half cubic centimeter quantities of each in small agglutination tubes. These were incubated at 37.5° C. for one hour in the thermostat and then kept at ice-box temperature over night.

In order to keep the conditions for all the tests uniform, and to eliminate the slight error which might occur in making several series of serum dilutions, all the typhoid antigens were set up with one series of dilutions. The emulsions were made up to a uniform turbidity in all cases. The bacteria were grown upon neutral agar slants. (Several control tests were made to determine the effect of acidity and alkalinity upon the agglutination reaction. Two sera, one part inactivated at 56° C. for one-half hour and the other not inactivated, and two antigens were used. From the results obtained we concluded that only in neutral solutions can constant results be obtained. One per cent alkalinity retarded the reactions, while one per cent acidity accelerated them.)

The agglutinations carried out with nineteen typhoid strains were done before the more recently acquired organisms had been isolated or received. The following is a representation of the results obtained:

TABLE II.

Agglutination of typhoid strains in typhoid serum.

Strains.	P. & S. Serum.		R.I. Serum.		Novy Serum.	
	Titer.	Reaction.	Titer.	Reaction.	Titer.	Reaction.
B. typhosus Novy . . .	1/2000	+++	1/4000	++++	1/2000	++++
" 31 K	1/16000	+++	1/4000	++++	1/2000	++++
" 21 K	1/16000	+++	1/4000	++++	1/2000	++++
" 27 K	1/16000	+++	1/4000	++++	1/2000	++++
" 36 K	1/16000	+++	1/4000	++++	1/2000	++++
" 34 K	1/16000	+++	1/4000	++++	1/2000	++++
" Bull	1/16000	+++	1/4000	++++	1/2000	++++
B. typhosus P. & S. . . .	1/16000	++++	1/4000	++++	1/500	+
" Norris . . .	1/16000	++++	1/4000	++++	1/500	+
" No. 2 W.P.	1/16000	++++	1/4000	++++	1/500	+
" No. 12 W. .	1/16000	++++	1/4000	++++	1/500	+
" No. 1 W.P.	1/16000	++++	1/4000	++++	1/500	+
" R.I.	1/16000	++++	1/4000	++++	1/500	+
" Z.	1/16000	++++	1/4000	++++	1/500	+
" Han. W. . .	1/16000	++++	1/4000	++++	1/500	+
" 28 K	1/16000	++++	1/4000	++++	1/500	+
B. typhosus Geary . . .	1/16000	++++	1/4000	++++	*	—
" Rawlings .	1/16000	++++	1/4000	++++	1/100	+
B. typhosus Plosser . .	—	—	—	—	—	—

* Negative in a serum dilution of 1/50.

The second and third groups differed from the first in the character of the reaction on B. typhi abdominalis P. & S. serum, and also in both the titer and intensity of the reaction on B. typhi abdominalis Novy serum. The second group differed from the third in its very marked difference on the B. typhi abdominalis Novy serum. The fourth group is represented by the non-agglutinating, non-motile B. typhi Plosser strain.

Such non-agglutinating types may have been previously

isolated from water supplies. Thus Williams,[7] after a careful examination of the literature, was able to find only six authentic cases in which a typical typhoid bacillus isolated from water supply was confirmed by agglutinations. Stokes and Hachtel[8] added a seventh to this number. I have recently isolated two more such non-agglutinating typhoid strains from the stools of normal individuals. These are at present being studied.

It may be argued that one or two strains cannot make a group, yet when the variation is such a primary one as absolute inagglutinability and lack of motility as in the Plosser strain, it may be a type which in our search for B. typhosus we fail to recognize because of the absence of those characteristics, the presence of which are often the basis, and too often the only basis, of identification in routine work.

It is difficult to support a classification or to make one on the basis of the agglutination reaction, and we simply offer this grouping as a convenient means of presentation to show that differences occur. Where the differences are so slight, as variations in turbidity, results can be judged with difficulty. The variations in titer of different antigens upon the same serum is, however, a more basic criterion, but even there the reaction cannot be accepted as conclusive, since two antigens reacting up to the same dilution in the same serum may be totally different. Thus Castellani[9] found that a B. coli strain that he was working with and a B. paradysentery gave almost the same reaction — (1/400–1/800), with a B. typhi abdominalis serum. He found that for a more correct determination of the antigenic nature of an organism serum adsorptions were necessary.

That agglutination is not sufficiently specific to be used in classifying the antigenic properties of a single group of organisms is shown by Durham's[10] hypothetical reasoning. It may be well to quote directly from his paper. He says:

"In order to explain the somewhat perplexing partial and mutual reactions of agglutinating sera upon different groups or races of bacteria, the following graphic method may be suggested. I suppose that a given

agglutinin is not a single substance, but a complex one, the constituent elements of which I will designate by capital letters, while the bacillary components which are capable of giving rise to the formation of agglutinins (when introduced into an animal) may be represented by the corresponding small letters. Thus if we take typhoid and enteritidis sera, which give some mutual reaction, they may be represented thus:

Elements Concerned with Agglutinins.	B. Typhi.	B. Enteritidis (Gärtner).	B. Enteritidis (Hatton).
Serum constitution	A, B, C, D, E.	C, D, E, F, G, H.	E, F, G, H, J, K.
Bacillary constitution	a, b, c, d, e.	c, d, e, f, g, h.	e, f, g, h, j, k.

When typhoid serum containing A + B + C + D + E is added to typhoid culture a + b + c + d + e the maximum effect of clumping is produced where each substance reacts with the other; when it is added to Gärtner bacilli c + d + e + f + g + h it will only produce an effect when the substances, C, D, and E are able to affect a certain portion of the bacilli, or rather their constituents c, d, and e to a sufficient degree, still greater must be the concentration of the typhoid serum to produce an effect upon the bacilli containing only the susceptible substance c.

The matter is further complicated: for instance, it may be supposed that although two typhoid races both consist of a + b + c + d + e, *these substances are not present in equal quantities*. Thus one typhoid race or individual bacillus may be represented by the formula 20a + 10b + 5c + 2d + 1e, and another by 1a + 2b + 5c + 10d + 20e. When tested by a third serum *containing all the constituents in equal quantity*, *i.e.*, (A = B = C = D = E) they may give identical dilution limits, but they will not do so when tested by means of their own sera."

It is apparent from this reasoning that agglutination, though giving a sufficient basis upon which to judge the presence or absence of antigenic differences, cannot serve as the most exact basis of classification, and that adsorption is far superior for this purpose.

Gruber[11] found that typhoid serum lost its agglutinating property after agglutinating B. typhosus. Hahn and Trommsdorff[12] came to the same conclusion. Castellani applied this method to his study of the group reactions existing between B. typhosus, B. coli, and B. paradysentery, and in a paper which is classical for its clearness he has proved the value of serum adsorptions as a means of studying antigenic properties.

Adsorption reactions. — A preliminary test was carried out to determine the value of agglutinin adsorption out of typhoid sera with typhoid antigen as a basis of classification. A well-controlled technic was necessary to insure maximum possible adsorption. The sera were diluted to one-eighth of the highest dilution in which agglutination occurred with the homologous strain. This made it possible to test for agglutinins in the adsorbed serum in a dilution sufficiently strong to give a sharp reaction whenever no adsorption occurred, and still not too strong to prevent all the agglutinins being readily adsorbed out by the homologous and similar strains. Thus, in a serum that had a titer of 1/16000, an initial dilution of 1/2000 was made. One-half cubic centimeter of this was adsorbed with a half cubic centimeter of a heavy typhoid antigen emulsion in isotonic salt solution, the half cubic centimeter representing the full growth on an agar slant. This was incubated at 37.5° C. from two to four hours, and kept at ice-box temperature for four days. Whenever the supernatant fluid became clear, dry antigen was added, and the tube again incubated. The organisms were then centrifugalized out, and a half cubic centimeter of the supernatant fluid, now in a dilution of 1 4000, was tested for agglutinating antibodies with the homologous strain. This gave a final dilution of 1/8000. Whenever there was a partial or incomplete adsorption, a sharp agglutination took place.

The preliminary test was carried out by adsorbing each of the four sera with the four homologous antigens. The reactions were sharp and clearly showed that, using only the four sera and their homologous strains, B. typhosus can be divided into distinct groups on the basis of the adsorption reactions. Table III. is a graphic representation of the results obtained:

TABLE III.

Adsorbing Antigens.	Sera.			
	B. Typhosus R.I.	B. Typhosus Rawlings.	B. Typhosus P. & S.	B. Typhosus Novy.
B. typhosus R.I.	+	+	+	+
" Rawlings	+ ?	+	+	±
" P. & S.	±	+	+	–
" Novy	–	–	–	+

The plus sign represents complete adsorption and no agglutination with the homologous strain; the minus sign represents agglutination with the homologous strain after attempted adsorption with the indicated antigen, which, therefore, indicated incomplete adsorption; and the plus-minus sign indicates almost complete adsorption.

Speaking in terms of the agglutinin content of the sera, it is evident from this preliminary test that B. typhosus R.I. contains all the agglutinins of the three other sera, that B. typhosus Rawlings is very close to it, though not exactly similar, in that the Rawlings antigen fails to give an absolutely complete adsorption of the Novy serum, that B. typhosus P. & S. contains the greater part, but not all of the B. typhosus R.I. agglutinins, and that it contains fewer of the Novy agglutinins than Rawlings does, and that B. typhosus Novy contains a minimum amount of the agglutinins common to the other three. The most noticeable feature of these tests is the fact that the B. typhi abdominalis Novy strain seems to stand apart as a definitely differentiable type.

Since these preliminary tests had shown so definitely that sharp antigenic differences could be observed among culturally identical typhoid bacilli, it, of course, became imperative to extend our observations to include a larger number of strains.

This work is still going on. As far as it has been carried up to the present time, it is tabulated in the following protocol:

TABLE IV.

Adsorbing Antigen.	Sera. B. Typhosus R.I.	B. Typhosus Rawlings.	B. Typhosus P. & S.	B. Typhosus Novy.
B. typhosus R.I.	+	+	+	+
" Han. W.	+	+	+	+
" No. 12 W.	+	+	+	+
" Rawlings*	+ ?	+	+	+
" Geary	+	+	+	+
" 21 K	+	+	+	+
" 27 K	+	+	+	+
" 28 K	+	+	+	+
" 31 K	+	+	+	+
" 34 K	+	+	+	+
" 36 K	+	+	+	+
" 1 W.P.	+	+	+	+
" 2 W.P.	+	+	+	+
" Ruby	+	+	+	+
" Schaffer	+	+	+	+
" Moore s.f.	+	+	+	+
" Hint	+	+	+	+
" Moore bl.	+	+	+	+
" Ryan	+	+	+	+
B. typhosus Zinsser	+	+	+	—
" Bull.	+	+	+	—
" Norris	+	+	+	—
" P. & S.	Almost complete adsorption.	+	+	—
B. typhosus Roth	+	±	—	+
B. typhosus Dohaney	±	+	—	—
" Cornwell	±	+	—	—
B. typhosus Novy	—	—	—	+
B. typhosus Felix	—	—	—	—
" Army	—	—	—	—
B. typhosus Plosser †	—	—	—	—

* Though B. typhosus R.I. and B. typhosus Rawlings are considered members of the same group, it is evident from our preliminary adsorption test, and it repeats itself here in the adsorption reactions of the "Dohaney" and "Cornwell" strains, that a slight difference does exist between them, since these strains do not give complete adsorption of "R.I." serum while they adsorb "Rawlings" serum completely. However, the difference is too slight to warrant separating them.

† B. typhosus Plosser is a non-motile, non-agglutinating organism, and differs in these respects from the "Felix" and "Army" strains. Two similar non-agglutinating strains have been recently isolated from the stools of normal individuals.

A graphic representation of these divisions, and the number of strains included in each, and their agglutination and adsorption reactions is given in the following table:

TABLE V.

Group.	Number of Strains.	Agglutination in Typhoid Serum.	Adsorption in Sera.			
			Typhosus R.I.	Typhosus Rawlings.	Typhosus P. & S.	Typhosus Novy.
A	19	+	+	+	+	+
B	4	+	+	+	+	−
C	1	+	+	±	−	+
D	2	+	±	+	−	−
E	1	+	−	−	−	+
F	2	+	−	−	−	−
G	3	−	−	−	−	−

In the foregoing we have shown that typhoid bacilli, formerly believed to be an entirely homogeneous group represented by one cultural and antigenic type, can be divided into sub-groups, both by cultural and serological methods.

Having determined this it was, of course, of importance to ascertain how the cultural subdivisions conformed with those found on the basis of serum tests. The study of the relationship furnished proof of the parallelism of biological activity and antigenic property. Thus the organisms which fell into antigenic groups A, B, C, and E, occurred, with the exception of two strains in A, in cultural type No. 1. The organisms of antigenic groups D and F fall into cultural type No. 2. Likewise, cultural type No. 3 is a distinct antigenic group. A combination of antigenic groups and cultural types yields eight separate divisions. These are shown in Table VI.:

TABLE VI.

Strain.	Cultural Type.	Antigenic Group.
B. typhosus R.I.	No. 1.	A.
" Han. W.		
" No. 12.		
" Geary.		
" 28 K.		
" 34 K.		
" 36 K.		
" No. 1 W.P.		
" Ruby.		
" Schaffer.		
" Moore s.f.		
" Hint.		
" Moore bl.		
" Ryan.		
" 21 K.		
" 27 K.		
" 31 K.		
B. typhosus Zinsser.	No. 1.	B.
" Norris.		
" Bull.		
" P. & S.		
B. typhosus Roth.	No. 1.	C.
B. typhosus Novy.	No. 1.*	E.
B. typhosus Rawlings.	No. 2.	A.
" No. 2 W.P.		

* Fermented mannite slowly.

TABLE VI.— *Continued.*

Strain.	Cultural Type.	Antigenic Group.
B. typhosus Dohaney. " Cornwell.	No. 2.	D.
B. typhosus Felix. " Army.	No. 2.	F.
B. typhosus Plosser.	No. 3.	G.

To complete this work it was considered desirable to study the group reactions of B. typhosus on one B. paratyphoid α, three B. paratyphoid β, and one B. typhi murium serum agglutinatively to determine to what dilution the group reaction occurred, and where it stopped and the typhoid reaction became specific. The sera titrated from 1/16000 to 1/32000. Eleven strains were used. The results are given in Table VII.:

TABLE VII.

Group reaction of B. typhosus on paratyphoid and typhi murium sera.

Typhoid Strains.	Para. α Serum.	Para. β Serum No. 1.	Para. β Serum No. 2.	Para. β Serum No. 3.	Typhi Murium Serum.
B. typhosus Zinsser	1/1000	1/2000	1/2000	1/400	1/1000
" Geary	1/200	1/200	. . . .	1/200	1/1000
" Plosser	Negative.	Negative.	Negative.	Negative.	Negative.
" Han. W. . . .	1/200	1/400	. . . .	1/100	1/200
" Novy	1/400	1/200	1/40	1/100	1/400
" Rawlings . . .	1/1000	1/1000	. . . .	1/100	1/1000
" Bull	1/1000	1/4000	1/2000	1/2000	1/1000
" P. & S.	1/400	1/2000	1/400	1/200	1/1000
" No. 12 W. . .	1/400	1/400	1/20	1/100	1/1000
" Norris	1/2000	1/2000	1/2000	1/2000	1/2000
" R.I.	1/400	1/200	1/40	1/100	1/200

The variations of the strains upon the individual sera was marked, but it was well shown that each strain had either a high or a low group reaction, regardless of which serum it was in. Thus the several strains showed group reactions in paratyphoid α serum that went as high as 1/200 to 1/1000 with a plus and minus reaction in one case in 1/2000, on paratyphoid β serum it ranged from 1/100 to 1/4000, and in B. typhi murium it ranged from 1/100 to 1/1000 with a plus and minus reaction in one case in 1/2000. On the basis of this we would suggest that wherever macroscopic agglutinations in tubes are made that no reaction be considered specific unless clumping takes place very rapidly in a dilution above 1/1000.

A relationship between the prozone and the group reaction was shown. In every case where the reaction went above 1/1000 a prozone occurred. In no case was a prozone evident where the group reaction went no higher than 1/200 to 1/400. Furthermore, it was evident that the best reaction did not occur in the lower dilutions, but in those intermediate between the lowest and highest in which agglutination occurred.

Summary of Part I. — From the work recorded we may conclude:

(I.) That B. typhosus can be divided into various subgroups, which differ from one another culturally and antigenically.

(II.) That the best method of antigenic investigation at present available is the adsorption method.

This work is not complete, and a final grouping must be postponed until a great many more strains from every available source can be collected. Also a great many more sera for adsorption reactions, and a larger variety of media will be necessary. It is sufficient for our present purpose if we have shown that such differences occur. The investigation of many strains is now being continued.

We wish also to emphasize that on a basis of these results

we believe that in typhoid prophylaxis the single "Rawlings" vaccine may not give complete protection. Recent cases of typhoid fever among soldiers who have received the prophylactic treatment have come to our attention, and lend weight to our belief. We would recommend that a polyvalent vaccine, or one made from an organism which is more representative of the whole group, should be used.

Furthermore, the experiments reported in this paper may explain why typhoid cases will occasionally be found to give no Widal reaction with the laboratory strains ordinarily employed.

SECTION II.

PARATYPHOID α. — The first classification of the pathogenic paratyphoid organisms was made by Schottmüller[13] in 1900. Cultural and antigen studies, which he carried out with strains isolated from five cases of fever which resembled typhoid clinically, showed that they could be divided into two groups. One of them, of which the "Müller" strain is typical, he classified as paratyphoid α; the other, of which the "Seeman" strain is typical, he classified as paratyphoid β. Both these strains are included in our collection. Gwyn,[14] two years earlier, had isolated an organism from the blood of a patient who presented all the symptoms of typhoid fever. The cultural characteristics were similar to those of the Gärtner organism, and he called it a "paracolon" bacillus. This strain is a typical paratyphoid α, and has come down to us as "paratyphoid Gwyn." It is, therefore, the first known isolation of the paratyphoid α organism. After Schottmüller's investigation similar cases were reported by Cushing,[15] Kurth,[16] Buxton and Coleman,[17] Libman,[18] and others.

We have considered as members of the paratyphoid α group only those organisms which give a specific agglutination above the range of group reaction on paratyphoid α serum.

In the group of paratyphoid α organisms here reported

are included the two earliest isolations, Gwyn and Schottmüller Müller. The strains used and their sources are given in Table VIII.:

TABLE VIII.

Sources of the B. paratyphoid a strains.

B. paratyphosus *a*	(K)	Obtained from Krumwiede.
"	(Army)	P. & S. collection.
"	(No. 16 K L)	Schottmüller Müller.
"	(No. 322 K L)	Isolated at Boston Board of Health, 1903.
"	(No. 166 K L)	University of Chicago: Strain No. I. (See paper in Jour. Infectious Diseases by Scott & Weller.)
"	(No. 167 K L)	University of Chicago: Strain No. II. (See paper in Am. Med. Ass., 1902.)
"	(No. 168 K L)	University of Chicago; Strain No. III. (Received from Buxton, Cornell Medical School.)
"	(No. 294 K L)	Isolated from blood at Hygienic Laboratory, 1907.
"	Gwyn	P. & S. Collection.
"	(55 K)	Isolated by Buxton in 1903.
"	(228 K)	Isolated from stool at N.Y. Board of Health, 1916.
"	(102 K)	Mt. Sinai o from Teague.
"	Regan	Isolated from blood of soldier at P. & S., 1916.
"	Barshow	Isolated from blood of soldier at P. & S., 1916.
"	Erickson	Isolated from blood of soldier at P. & S., 1916.
"	Connell	Isolated from stool of soldier at P. & S., 1916. Paratyphoid carrier.
"	Smith	Isolated from blood of soldier at P. & S., 1916.
"	Hyde	Isolated from stool of soldier at P. & S., 1916. Paratyphoid carrier.
"	Shannon	Isolated from blood of soldier at P. & S., 1916.
"	Yale	Isolated from stool of soldier at P. & S., 1916.

At present we can report upon twelve of these twenty strains. The work is being extended to include the others which were recently isolated. They will form part of a future communication.

The cultural and antigenic investigation showed that paratyphoid α strains differ from one another in both respects, and that a parallel may exist between them. Culturally the group is being studied on the same thirteen differential media that were spoken of under B. typhosus. The eight there reported can also be reported here. These are dextrose, mannite and maltose, lactose and saccharose, xylose, rhamnose and inosite.

Cultural reactions. — Dextrose: In dextrose broth it was found that eleven strains gave good gas and acid production in twenty-four hours, while one, No. 167, required forty-eight hours to ferment it sufficiently for a positive reading.

Mannite: In mannite the same eleven strains previously typical gave acid and gas in twenty-four hours, while No. 167 fermented with acid production only. It produced gas after forty-eight hours.

Maltose: Ten strains fermented maltose giving acid and gas. Two strains, one the previously atypical No. 167 and the other No. 32, failed to produce gas until the second day.

Rhamnose: All the strains produced acid and slight gas in twenty-four hours, and plentiful gas in forty-eight hours.

Lactose and saccharose: On lactose and saccharose all the strains were uniformly negative after prolonged incubation.

Xylose: All the strains were non-xylose fermenters, but a peculiarity of the xylose fermentation in this group, and one which repeats itself in the others, is that some of the strains produce a slight amount of gas without giving an acid reaction. Eight of our twelve strains did this. The use of xylose was suggested by Harding and Ostenberg,[19] and recently extended by Krumwiede, Pratt and Kohn,[20] who, in a recent issue of the Journal of Medical Research, have established the fact that the non-fermentation of xylose differentiates paratyphoid α and a part of the group which

we speak of as heterogeneous paratyphoid from the other members of the paratyphoid-enteritidis group.

Inosite: All the strains were uniformly negative on inosite.

A graphic representation of these results is given in Table IX.:

TABLE IX.

Cultural reactions of B. paratyphosus a.

<table>
<tr><th>Strain.</th><th>Dextrose.</th><th>Mannite.</th><th>Maltose.</th><th>Rhamnose.</th><th>Lactose.</th><th>Saccharose.</th><th>Xylose.</th><th>Inosite.</th></tr>
<tr><td>B. paratyphosus α (K).</td><td rowspan="11">Acid and gas, 24 hours.</td><td rowspan="11">Acid and gas, 24 hours.</td><td rowspan="10">Acid and gas, 24 hours.</td><td rowspan="12">Acid and slight gas, 24 hours. Plentiful gas, 48 hours.</td><td rowspan="12">Negative.</td><td rowspan="12">Negative.</td><td rowspan="12">Negative.*</td><td rowspan="12">Negative.</td></tr>
<tr><td>" (Army).</td></tr>
<tr><td>" (No. 16 K L).</td></tr>
<tr><td>" (No. 166 K L).</td></tr>
<tr><td>" (No. 168 K L).</td></tr>
<tr><td>" (294 K L).</td></tr>
<tr><td>" (Gwyn).</td></tr>
<tr><td>" (No. 55 K).</td></tr>
<tr><td>" (No. 228 K).</td></tr>
<tr><td>" (No. 102 K).</td></tr>
<tr><td>" (322 K L).</td><td>Gas, 2 days.</td></tr>
<tr><td>" (167 K L).</td><td>Gas, 2 days.</td><td>Gas, 2 days.</td><td>Gas, 2 days.</td></tr>
</table>

* Eight strains produced a very slight amount of gas.

The conclusions drawn are, that paratyphoid α: First — gives a uniformly negative reaction on lactose, saccharose, and inosite.

Second — that the large majority of strains ferment dextrose, mannite and maltose rapidly with gas and acid formation, but that there are atypical types which produce gas only on prolonged incubation.

Third — that they react uniformly in fermenting rhamnose slowly with acid and gas formation.

Fourth — that they do not produce acid on xylose, but the greater number of them produce a small amount of gas.

Antigenic reactions. — The antigenic reactions of the twelve strains have so far been studied upon five sera, one a paratyphoid α, three paratyphoid β, and one typhi murium. Nine of them agglutinated as high as the homologous strain (1/16000) in a paratyphoid α serum, one agglutinated up to 1/8000, and another, No. 168, only up to 1/2000. This strain was typical culturally. The antigenic activity of a third could not be observed comparatively because of a spontaneous reaction. These results are shown diagramatically in Table X.:

TABLE X.

Agglutination reactions of B. paratyphosus a strains in B. paratyphosus a Army serum.

Strain.	Highest Dilution in which Agglutination Occurred.
B. paratyphosus a (K)	1/16000
" (Army)	1/16000
" (No. 16 K L)	1/16000
" (No. 322 K L)	1/16000
" (No. 167 K L)	1/16000
" (No. 166 K L)	1/16000
" (No. 168 K L)	1/2000
" (No. 294 K L)	1/16000
" (Gwyn)	1/16000
" (55 K)	Spontaneous agglutination.
" (228 K)	1/8000
" (102 K)	1/16000

Adsorption reactions. — Adsorbing this paratyphoid α serum with each of these strains showed that eleven of them adsorbed it out completely, while the one strain, No. 168, which gave a low agglutination failed to do so. After

adsorbing with this strain the homologous antigen was agglutinated almost immediately by the adsorbed serum and completely precipitated after thirty minutes in the thermostat, showing that the antigenic difference is very marked, and that its similarity to the other paratyphoid α strains is no greater than that which exists between some paratyphoid β and typhoid organisms. This strain also differed from the others in its group reaction on paratyphoid β Army serum. Whereas all the other strains showed good group agglutination, it showed none. The adsorption reactions are shown in Table XI.:

TABLE XI.

Adsorption of paratyphoid a Army serum with paratyphoid a strains.

Adsorbing Antigen.	Adsorption.
B. paratyphosus *a* (K)	+
" (Army)	+
" (16 K L)	+
" (322 K L)	+
" (166 K L)	+
" (167 K L)	+
" (294 K L)	+
" (Gwyn)	+
" (55 K)	+
" (228 K)	+
" (102 K)	+
B. paratyphosus *a* (168 K L)	—

The conclusion drawn from this work is that there are at least two antigenically different paratyphoid α types.

The group reaction of paratyphoid α antigen with paratyphoid β serum varied considerably with both. On one serum the two slow-fermenting types, Nos. 167 and 322,

agglutinated in a 1/2000 dilution, while none of the others went above 1/400. In a second serum No. 322 reacted in a 1/100 dilution, while none of the other strains gave a group reaction even as low as 1/20. In a third serum the same strain agglutinated in 1/200, while none of the others went above 1/100. It would appear from this that a relationship between antigenic properties, as shown by the group reaction, and biological activity may in some cases exist.

Table XII. is a graphic representation of the group reactions:

Table XII.

Group reaction of paratyphoid α on paratyphoid β and typhi murium serum.

Strain.	Sera.			
	Paratyphoid β No. 1.	Paratyphoid β Seeman.	Paratyphoid β Army.	B. Typhi Murium.
B. paratyphosus α (K) . . .	1/200	Negative.	1/100	Negative.*
" (Army) . .	1/400	"	1/100	"
" (16 K L) .	1/400	"	1/40	"
" (322 K L) .	1/2000	1/100	1/200	"
" (166 K L) .	1/400	Negative.	1/100	"
" (167 K L) .	1/2000	"	1/100	"
" (168 K L) .	1/400	"	Negative.	"
" (294 K L) .	1/400	"	1/100	"
" (Gwyn) . .	1/400	"	1/100	"

* Negative represents no agglutination in a 1/10 dilution of the serum.

Of all the groups included in this research paratyphoid α appears to have the most individual or specific antigenic properties. A comparison of the various group reactions showed that the other groups gave their lowest agglutination on paratyphoid α serum, and that paratyphoid α antigen gave the lowest reaction on the other sera.

Paratyphoid β. — The twenty-five paratyphoid β strains included in this part of the study are all of undoubted human origin, and were isolated from cases of clinical paratyphoid fever. A list of the cultures and their sources is given in Table XIII.:

TABLE XIII.

Sources of the B. paratyphosus β strains.

B. paratyphosus β	(No. 1 P. & S.)	P. & S. collection.
"	(No. 395 K L)......	Isolated from blood, 1907.
"	(Seeman)	P. & S. collection.
"	(96 K)	—— —— —— ——
"	(Army)	P. & S. collection.
"	(No. 22 K L).......	Schottmüller Seeman.
"	(No. 323 K L)......	Isolated at Boston Board of Health, 1903.
"	(No. 169 K L)......	University of Chicago (Strain IV.).
"	(No. 13 R)	Isolated from blood at Roosevelt Hospital.
"	(No. 6 R)	Original St. Luke's strain; obtained from Roosevelt Hospital.
"	(No. 7 R)	Original St. Luke's strain; obtained from Roosevelt Hospital.
"	(No. 52 R)	Isolated from blood at Roosevelt Hospital, 1916.
"	(No. 9 R)	Original Bellevue strain; obtained from Roosevelt Hospital.
"	(No. 8 R)	Original Bellevue strain; obtained from Roosevelt Hospital.
"	(No. 236 K L)......	—— —— —— ——
"	(No. 324 K L)......	—— —— —— ——
"	(Kane)	Isolated from stool at Roosevelt Hospital, 1916.
"	(Rifkin)	Isolated from blood at Roosevelt Hospital, 1916.
"	(Gearing)	Isolated from blood at Roosevelt Hospital, 1916.
"	(No. 32 R).........	Isolated at Roosevelt Hospital from case of Dolan, 1916.
"	(No. 31 R).........	Isolated at N.Y. Department of Health, 1915.
"	(No. 222 K)........	Isolated from stool at N.Y. Department of Health, 1916.
"	(No. 223 K)........	Isolated from stool at N.Y. Department of Health, 1916.
"	(No. 224 K)........	Isolated from stool at N.Y. Department of Health, 1916.
"	(No. 56 K).........	Isolated at Johns Hopkins Hospital, 1898.

Culturally and antigenically their reactions were studied upon the same media and sera spoken of under paratyphoid α. As was the case in that group, here too, differences in both were found.

Cultural reactions. — The cultural reactions of the twenty-five strains used are tabulated in Table XIV.:

TABLE XIV.

Cultural reactions of strains of B. paratyphosus β.

<table>
<tr><th>Strain.</th><th>Dextrose.</th><th>Mannite.</th><th>Maltose.</th><th>Rhamnose.</th><th>Lactose.</th><th>Saccharose.</th><th>Xylose.</th><th>Inosite.</th></tr>
<tr><td>B. paratyphosus β (No. 52 R).</td><td rowspan="18">Acid and gas, 24 hours.</td><td rowspan="18">Acid and gas, 24 hours.</td><td rowspan="17">Acid and gas, 24 hours.</td><td rowspan="19">Acid and gas, 24 hours.</td><td rowspan="19">Negative.</td><td rowspan="19">Negative.</td><td rowspan="19">Acid and gas, 24 hours.</td><td rowspan="19">Acid and gas, 24 hours.</td></tr>
<tr><td>" (No. 9 R).</td></tr>
<tr><td>" (Kane).</td></tr>
<tr><td>" (Rifkin).</td></tr>
<tr><td>" (Gearing).</td></tr>
<tr><td>" (No. 32 R).</td></tr>
<tr><td>" (No. 1 P. & S.).</td></tr>
<tr><td>" (Seeman).</td></tr>
<tr><td>" (Army).</td></tr>
<tr><td>" (No. 22 K L).</td></tr>
<tr><td>" (No. 169 K L).</td></tr>
<tr><td>" (No. 13 R).</td></tr>
<tr><td>" (No. 6 R).</td></tr>
<tr><td>" (No. 222 K).</td></tr>
<tr><td>" (No. 223 K).</td></tr>
<tr><td>" (No. 224 K).</td></tr>
<tr><td>" (No. 31 R).</td></tr>
<tr><td>" (No. 7 R).</td><td>Gas, 48 hrs.</td></tr>
<tr><td>" (No. 8 R).</td><td>Gas, 48 hrs.</td><td>Gas, 48 hrs.</td><td>A.&G., 24 hrs.</td></tr>
<tr><td>B. paratyphosus β (No. 295 K L).</td><td>A.&G., 48 hrs.</td><td>A.&G., 48 hrs.</td><td>A.&G., 4 days.</td><td rowspan="5">Acid and gas, 24 hours.</td><td rowspan="6">Negative.</td><td rowspan="6">Negative.</td><td rowspan="5">Acid and gas, 24 hours.</td><td rowspan="6">Negative.</td></tr>
<tr><td>" (No. 96 K).</td><td rowspan="5">Acid and gas, 24 hours.</td><td rowspan="5">Acid and gas, 24 hours.</td><td>A.&G., 24 hrs.</td></tr>
<tr><td>" (No. 323 K L).</td><td>A.&G., 24 hrs.</td></tr>
<tr><td>" (No. 236 K L).</td><td>A.&G., 48 hrs.</td></tr>
<tr><td>" (No. 324 K L).</td><td>A.&G., 3 days.</td></tr>
<tr><td>" (No. 56 K).</td><td>A.&G., 3 days.</td><td>A.&G., 48 hrs.</td><td>A.&G., 48 hrs.</td></tr>
</table>

The dextrose and mannite fermentations were uniform. Twenty-three of the strains gave acid and gas in twenty-four hours. Two strains fermented slowly, requiring forty-eight hours for a positive reaction. On maltose five strains gave a delayed fermentation of from two to four days. It is significant to note that four of these form an antigenic group. On rhamnose and xylose all the strains gave acid and gas production in twenty-four hours, with the exception of one (No. 56 K), which produced gas after two days. There was a marked difference in the degree of acid and gas formation on rhamnose. Twenty-one of the strains gave acid with very little gas, which did not increase on further incubation. Three strains produced acid and gas very powerful in eighteen hours. The reactions on lactose and saccharose were uniformly negative.

Of the twenty-five strains used, nineteen gave acid and gas on inosite in eighteen hours. The other six were negative after ten days' incubation. It has been noted by many observers that the intensity of the agglutination reaction varies considerably with different strains. This has been our experience, and we believe that we have parallelled the positive and negative gas formation upon inosite of the paratyphoid β strains with their agglutinating properties. Of the six strains which gave no gas on inosite, four gave a turbid agglutination. Of the other two, one agglutinated spontaneously almost completely and one slightly, thus preventing their antigenic activity being observed. The emulsions were made up to a uniform turbidity in all cases. Owing to the variations in agglutination of different strains, probably due to their varying sensitiveness to electrolytes, and the technical difficulties encountered in obtaining uniform conditions, it is difficult to support a cultural difference on a basis of the intensity of the reaction. The adsorption reactions showed, however, that four of these six non-inosite fermenting strains form an antigenic group.

Agglutination reactions. — Considerable difference was found between the various strains in their agglutination on paratyphoid β sera. Kutcher and Meinicke[21] had previously found agglutinative differences in paratyphoid β on typhi murium serum. One group of their paratyphoid β strains agglutinated up to the titer of the serum, while another went only as high as the group reaction with typhoid serum. Intermediates occurred between these two. Our differences occurred not only in the character of the reaction, but in the final dilution in which the reaction took place. On four sera the greater number agglutinated as high as the homologue, several agglutinated lower than this, and one strain (No. 169), which was found by adsorption to be a distinct type, agglutinated on two sera whose titer was 1/16000 only up to a dilution of 1/2000.

The group reaction on paratyphoid α sera went in no case above a dilution of 1/200.

Very good agglutination occurred in B. typhi murium serum, several of the strains reacting as high as the homologue.

Adsorption reactions. — All of the paratyphoid strains were used to adsorb out two paratyphoid β sera, one Army, the other Schottmüller Novy Seeman, and one B. typhi murium serum. In no case did complete adsorption of the B. typhi murium serum occur. After the adsorbing antigen was centrifugalized off and the homologous strain added, it agglutinated almost immediately. The reactions are given in Table XV.:

TABLE XV.

Adsorption reactions of paratyphoid β sera with paratyphoid β antigens.

Strains.	Sera. B. Paratyphosus β Army.	B. Paratyphosus β Seeman.	B. Typhi Murium.
B. Paratyphosus β (No. 295 K L) . . .	—	—	—
" (No. 236 K L) . . .	—	—	—
" (No. 324 K)	—	—	—
" (No. 56 K)	—	—	—
B. Paratyphosus β (No. 323 K)	±	—	—
B. Paratyphosus β (No. 1, P. & S.) . .	±	±	—
B. Paratyphosus β (No. 22 K L) . . .	+	±	—
" (No. 8 R)	+	±	—
B. Paratyphosus β (No. 169 K L) . . .	—	+	—
B. Paratyphosus β (No. 6 R)	+	—	—
" (No. 31 R)	+	—	—
" (No. 223 K)	+	—	—
B. Paratyphosus β (Seeman)	+	+	—
" (No. 96 K)	+	+	—
" (Army)	+	+	—
" (No. 13 R)	+	+	—
" (No. 7 R)	+	+	—
" (No. 52 R)	+	+	—
" (No. 9 R)	+	+	—
" (Kane)	+	+	—
" (Rifkin)	+	+	—
" (Gearing)	+	+	—
" (No. 32 R)	+	+	—
" (222 K)	+	+	—
" (224 K)	+	+	—

The adsorption reactions on the two paratyphoid sera showed seven antigenic types or groups. Four of these are distinct, the others are apparently intermediates. Thus,

speaking of paratyphoid β Army serum and paratyphoid β Schottmüller Novy Seeman serum as Nos. 1 and 2 respectively, and using plus to designate adsorption and minus to designate no adsorption and agglutination with the homologous strain, and plus and minus to designate a partial agglutination or adsorption which is not complete we have:

TABLE XVI.

Grouping of paratyphoid β on a basis of the adsorption reactions.

Group.	Number of Strains.	No. 1.	No. 2.
1	4	—	—
2	1	±	—
3	1	±	±
4	2	+	±
5	1	—	+
6	3	+	—
7	13	+	+

The members of Group No. 1 are uniform in their reaction on inosite and maltose, which, with the exception of two strains on inosite Nos. 96 and 323, and another on maltose No. 7 R, is characteristic of this group.

Group No. 2, represented by the single strain No. 323, seems to possess the same inosite non-fermenting property that Group No. 1 has.

Strain No. 169, the only culture which gave a better adsorption of serum No. 2 than serum No. 1, agglutinated up to 1/2000 in paratyphoid β sera, while the other strains went up to 1/8000 to 1/16000.

Conclusions. — The cultural reactions show that:

First — paratyphoid β consists of an inosite fermenting and a non-inosite fermenting group.

Second — that this group corresponds with the agglutination reactions.

Third — that the non-inosite fermenting group is also atypical on maltose.

Furthermore, we have at least seven antigenic groups of paratyphoid β among our strains, four of which are sharply distinct while three may be intermediate.

An apparent contradiction occurs in the adsorption study. It is that paratyphoid β Army antigen adsorbs out paratyphoid β Seeman serum and paratyphoid β Seeman antigen adsorbs out paratyphoid β Army serum, yet, when these two sera are adsorbed by other paratyphoid β antigens, they react differently. This appears contradictory on the basis of the Ehrlich "antigen receptor" and "serum agglutinin" idea, but simply gives another example of the failure of the theory to fit the facts.

B. TYPHI MURIUM. — Five strains of B. typhi murium from widely divergent sources were used. The organisms were in every case isolated from mouse typhoid. This group has not been differentiated from human paratyphoid β, hog cholera, or B. abortus, and the prevailing opinion seems to be that they are all the same type, but coming from different sources.

Cultural reactions. — Dextrose, mannite, maltose, lactose, and saccharose: Culturally, all the strains were typical in dextrose, mannite, maltose, lactose, and saccharose broth.

Xylose: The xylose fermentation divided the five strains into two groups; the first, consisting of three strains, gave doubtful acid and gas production until the fourth or fifth day, when the reaction became sufficiently marked to be considered positive, while the other two strains gave immediate fermentation. These two groups are antigenically distinct also, the first agglutinating in typhi murium and paratyphoid β serum, while the other is non-agglutinating in either of these.

Inosite: On inosite agar two strains gave gas, while three did not. Of the latter, two were the members of the non-agglutinating group.

Rhamnose: In rhamnose broth all the strains gave acid and gas in twenty-four hours.

The cultural reactions are shown in Table XVII.:

TABLE XVII.

Cultural reactions of strains of B. typhi murium.

Strains.	Dextrose.	Mannite.	Maltose.	Rhamnose.	Lactose.	Saccharose.	Xylose.	Inosite.
B. typhi murium (No. 13).	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Negative.	Negative.	A. & G., 24 hours.	Negative.
" (K).	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Negative.	Negative.	A. & G., 24 hours.	Negative.
" (W).	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Negative.	Negative.	A. & G., 4–5 days.	Negative.
" (P. & S.).	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Negative.	Negative.	A. & G., 4–5 days.	A. & G., 24 hours.
" (No. 138 K).	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Acid and gas, 24 hours.	Negative.	Negative.	A. & G., 4–5 days.	A. & G., 24 hours.

Agglutination reactions. — Three strains agglutinated either as high as the homologue or only slightly lower on three paratyphoid β and one typhi murium serum. The other two did not. The group reaction in paratyphoid α serum went up to 1/200 in the agglutinating and 1/40 to 1/100 in the non-agglutinating strains.

TABLE XVIII.

Agglutination reaction of strains of B. typhi murium.

Strains.	Sera.				
	Para. β Seeman.	Para. β No. 1 P. & S.	Para. β Army.	Typhi Murium.	Para. α Army.
B. typhi murium (13)	+	+	+	+	1/200
" (K)	+	+	+	+	1/200
" (W)	+	+	+	+	0
" (P. & S.) . . .	—	—	—	—	1/40
" (138 K)	—	—	—	—	1/100

Adsorption reactions. — The three agglutinating B. typhi murium, the twenty-five paratyphoid β strains, six hog cholera, and two B. abortus cultures, were each used to adsorb out a typhi murium serum.

Complete adsorption was obtained with all the B. typhi murium strains. None of the paratyphoid, hog cholera, or abortus strains adsorbed out the B. typhi murium serum sufficiently to prevent a sharp agglutination with the homologue after ten minutes in the thermostat.

Table XIX.

Adsorption of B. typhi murium serum with B. paratyphosus β, B. choleræ suis, B. abortus, and B. typhi murium.

Groups.		Adsorption.
B. paratyphosus β	25 strains	—
B. choleræ suis	6 "	—
B. abortus	2 "	—
B. typhi murium (agg. group),	3 "	+

The conclusions are: First — that the agglutinating group of B. typhi murium is a specific class, and, though closely related antigenically to paratyphoid β, hog cholera, and B. abortus, it is antigenically distinct.

Second — that there are two groups of B. typhi murium, one an agglutinating, the other a non-agglutinating group.

Third — that the agglutinating group can be divided into two sub-groups, one inosite fermenting, the other non-fermenting.

Fourth — that the non-agglutinating group is non-inosite fermenting.

B. Choleræ suis. — Seven strains of B. choleræ suis were studied. As previously stated, this group has not yet been differentiated from B. paratyphosus β, B. typhi murium, and B. abortus.

The cultures and their sources are given in Table XX.:

TABLE XX.

Sources of B. choleræ suis strains.

B. choleræ suis	(No. 332 K L)	(Dorsett strain) Michigan Agricultural College.
"	(No. 239 K L)	Johns Hopkins University; obtained from Billings, 1907.
"	(No. 258 K L)	Parke, Davis & Co., Isolated from hog cholera, 1903.
"	(No. 334 K L)	Novy.
"	(No. 112 K)	Originally from Teague.
"	(No. 118 K)	From Board of Health Collection; source unknown.
"	(No. 120 K)	Originally from Dr. Zingher.

Cultural reactions. — Dextrose: On dextrose broth they produced acid and gas after two days' incubation, with the exception of one, No. 112, which failed to give gas.

Mannite and maltose: Five strains produced acid and gas on mannite and maltose. The other two, Nos. 112 and 334, were negative after prolonged incubation.

Lactose and saccharose: Lactose and saccharose fermentations were uniformly negative.

Xylose: The five strains typical on mannite and maltose gave a slow fermentation on xylose, producing acid after twenty-four hours and gas after three days; No. 334 remained negative, while No. 112 produced a slight amount of acid on the third day.

Rhamnose: Four strains gave acid on rhamnose and gas after prolonged incubation; the other three gave no gas.

Inosite: All the strains were uniformly negative on inosite agar. The reactions are tabulated in Table XXI.:

TABLE XXI.

Cultural reactions of B. choleræ suis strains.

Strains.	Dextrose.	Mannite.	Maltose.	Lactose.	Saccharose.	Xylose.	Rhamnose.	Inosite.
B. choleræ suis (No. 332 K L). " (No. 239 K L). " (No. 258 K L). " (No. 118 K).	Acid and gas, 2 days.	Acid and gas, 24 hours.	Acid and gas, 2 days.	Negative.	Negative.	Acid, 24 hours. Gas, 2–3 days.	Acid and gas, 2 days.	Negative.
B. choleræ suis (No. 112 K).	Acid.	Acid.	Acid.	Neg.	Neg.	Slight acid 3–4 days.	Acid.	Neg.
B. choleræ suis (No. 334 K L).	A. & G., 2 days.	A. & G., 5 days.	A. & G., 4–6 days.	Neg.	Neg.	Neg.	Acid, 4 days.	Neg.
B. choleræ suis (No. 120 K).	A. & G., 2 days.	A. & G., 2 days.	A. & G., 2 days.	Neg.	Neg.	A. & G., 3 days.	Acid.	Neg.

Very interesting cultural reactions are given by B. choleræ suis (112 K). With the exception of the rhamnose fermentation they are typical typhoid reactions, and, on the basis of this, one might exclude it from the hog cholera group. Yet it gives an agglutination in paratyphoid β serum almost as high as the homologous strain. It is to our knowledge the only non-gas producing organism which gives this antigenic reaction.

Agglutination reactions. — Six of the strains agglutinated well on paratyphoid β and typhi murium serum. One (No. 322 K L) did not agglutinate, though culturally typical.

TABLE XXII.

Agglutination of B. choleræ suis on paratyphoid β serum.

(Serum titer 1/16000.)

Strain.	Agglutination.
B. choleræ suis (332 K L)	Negative.
" (239 K L)	1/8000
" (258 K L)	1/8000
" (118 K)	1/8000
" (112 K)	1/8000
" (334 K L)	1/8000
" (120 K)	1/8000

Adsorption reactions. — Adsorptions of two paratyphoid β and one typhi murium serum were carried out, using the six agglutinating strains. The results were uniformly negative, the adsorbed sera agglutinating the homologous antigen in every case.

The conclusions drawn are: First — that B. choleræ suis reacts uniformly on lactose, saccharose, and inosite.

Second — that atypical types occur which ferment the other carbohydrates slowly, and, in the case of xylose, one strain failed to ferment it.

Third — that there are at least two groups based on antigenic properties, one agglutinating and one non-agglutinating group.

Fourth — that the agglutinating group of B. choleræ suis is antigenically distinct from B. typhi murium, and six of our seven groups of paratyphoid β. It is probably antigenically specific and distinct from all its co-agglutinators. A proof of this we hope to offer in an early paper.

B. ABORTUS. — The two strains of B. abortus which we used gave typical cultural and agglutinative reactions. This group has also been culturally and antigenically indistinguishable from B. paratyphosus β, B. typhi murium, and

B. choleræ suis. The failure to adsorb out B. typhi murium and paratyphoid β sera showed, however, that they differ antigenically from six of our seven groups of paratyphoid β and B. typhi murium.

HETEROGENEOUS PARATYPHOID STRAINS.—The twenty-one strains of paratyphoid included in this group came from a variety of sources, but in no case has proof been found that they are the cause of paratyphoid fever. Several of the cultures were stock laboratory strains which have no history. Isolations from stools were made in a case of nephritis, German measles, paratyphoid β, and pyelitis. Several strains were obtained from urine. Two isolations, one from the blood and the other from the urine, were made from a surgical case. Recently I have isolated eight more such strains, one from a paratyphoid α case, one from a dysentery case from which the bacillus Rosen was also isolated, and six from the stool of normal individuals.

Uhlenhuth and Hübener obtained an organism from a case of meat poisoning, which was similar to paratyphoid, but did not agglutinate in sera of the fixed types. This organism, which they called paratyphoid C., was agglutinated by the patient's serum and also by an enteritidis serum. Messerschmidt, Babes and Foedorasco, Arzt and Boese, and Sieffert and others have also found these paratyphoid-like types.

Our organisms reacted culturally like typical paratyphoid, and differ from the fixed types in their non-agglutination in paratyphoid α or β sera. They fail to show any cross-agglutination. Three sera made from strains belonging to this group reacted with the homologous antigen only. Culturally, they all produce acid and gas very actively on dextrose, mannite, and maltose, and are negative in lactose, saccharose, and inosite.

I have not included here B. enteritidis, B. columbensis, and the Danysz organism. B. columbensis came from Castellani, and was isolated from a case of paratyphoid fever in Ceylon. All these organisms are culturally like our heterogeneous paratyphoids, and, like them, fail to agglutinate in paratyphoid α or β sera above the range of group reaction.

Summary of Section II. — A comparison of the reactions of the members of these several paratyphoid and allied groups show:

That on dextrose broth paratyphoid α and β and also B. typhi murium and the heterogeneous paratyphoids give good gas and acid formation in twenty-four hours. A very few delayed fermentations occur. The hog cholera strains are much slower, requiring forty-eight hours for a positive fermentation, and in one case no gas was produced.

On mannite broth they all produce acid and gas in twenty-four hours, but all the groups, with the single exception of B. typhi murium, include atypical types; one paratyphoid α, two paratyphoid β, and two hog cholera strains which ferment slowly, and one hog cholera which gives no gas.

They produce acid and gas on maltose, but, as was the case with mannite, atypical types occur in all the groups but B. typhi murium. Two paratyphoid α strains and five paratyphoid β strains were slow, and the two hog cholera strains which were slow on mannite were also slow on maltose.

All the groups are uniformly negative on lactose and saccharose. On rhamnose broth they all give acid and gas, but the paratyphoid α strains require forty-eight hours for good gas formation, in the paratyphoid β group there was only one strain which reacted slowly, and in the B. choleræ suis group three gave acid without gas, while four produced gas very slowly. The B. typhi murium strains produce good acid and gas in twenty-four hours, and it is interesting to note that here, as in the sugars previously mentioned, no atypical reactions occurred.

On xylose, paratyphoid α is negative, while paratyphoid β, typhi murium, and B. choleræ suis (with the exception of one strain) ferment it with acid and gas production.

On inosite, paratyphoid α, B. choleræ suis, and B. abortus are negative, while paratyphoid β and B. typhi murium each have one fermenting and one non-fermenting group.

To summarize the antigenic findings they are:

That we have among our strains two groups of paratyphoid α; seven groups of paratyphoid β, four distinct and

three apparently intermediate; that B. typhi murium is composed of two antigenic groups, one agglutinating and one non-agglutinating. The agglutinating group is divided into two sub-groups by inosite fermentation; furthermore, this group can be differentiated from all the other agglutinating members of the several groups by adsorption of a B. typhi murium serum.

B. choleræ suis, like B. typhi murium, is composed of one agglutinating and one non-agglutinating group. On the basis of adsorption reactions it can be differentiated from all the other groups, with the single exception of four of our paratyphoid β strains and B. abortus.

B. abortus can, on the basis of adsorption, be differentiated from all our strains, with the exception of B. choleræ suis and four paratyphoid strains.

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BACTERIOLOGICAL OBSERVATIONS IN EXPERIMENTAL POLIOMYELITIS OF MONKEYS.*

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In a previous report[1] we stated briefly the cultural characteristics of a pleomorphic streptococcus isolated from the brain and cord of cases of human poliomyelitis. We have now studied the bacteriology of the central nervous system of ten monkeys that succumbed to experimental poliomyelitis.

Figure 1 shows graphically the history of this series of animals, of which eight were M. rhesus, one was M. sinicus, and one was Cerco pithecus sabeus. The virus was passed from one monkey to the next as a five per cent emulsion in normal salt solution of glycerinated or fresh brain and cord, or as a filtrate of such an emulsion. The animals were put under ether anesthesia, and a dose averaging one cubic centimeter was injected into the frontal region of one of the cerebral hemispheres. Monkeys 28, 40, and 46, which were injected with filtrate, received in addition to the intracerebral inoculation an injection of ten cubic centimeters of filtrate into the peritoneal cavity.

Each of these monkeys showed to a greater or less degree the usual symptoms of experimental poliomyelitis — excitement, tremor, and paralysis. Most of them were prostrate when killed, but two were etherized and autopsied when it became evident that they were recovering from abortive attacks of the disease. Microscopic sections of brain and cord gave the typical picture of experimental poliomyelitis in each of the animals.

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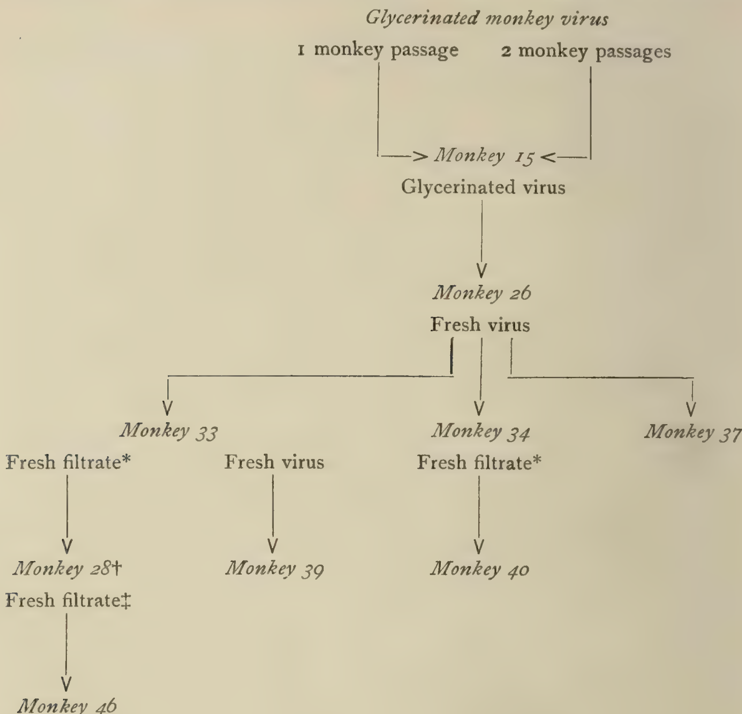
Figure 1.

DIAGRAM SHOWING DERIVATION OF MATERIAL USED TO PARALYZE 10 MONKEYS.



* Berkefeld N filter. † Previously inoculated with an inert virus.
‡ Dense porcelain candle.

The autopsies were done under rigid aseptic precautions. Emulsions ground up in a sterile air chamber, or fragments of the brain, cord, and intervertebral ganglia were transferred immediately into culture media.

Methods of cultivation. — Many types of culture media were employed successfully. Of these the following were the more important:

1. Ascitic-dextrose broth: made with Witte peptone and Liebig's extract of beef, titrated to .6 — .8 acid to phenolphthalein and containing dextrose .2 per cent and ascitic fluid ten per cent.

2. Ascitic broth: as above, without the dextrose.

3. Ascitic-dextrose agar: containing one per cent dextrose and 2.5 per cent agar.

4. Unheated and unfiltered ascitic fluid.

5. Steamed ascitic fluid.

6. One and five-tenths per cent agar (two parts) + ascitic fluid, ascitic-dextrose broth or ascitic broth (one part).

7. Plates made of agar and fresh human blood (ten per cent).

8. Slants of similar blood-agar; aërobic and anerobic.

The ascitic fluid and the ascitic agar were used in tall tubes to which fresh sterile rabbit kidney and a layer of sterile mineral oil were added, according to the methods described by Flexner and Noguchi.[2] The column of medium measured .8 centimeter in diameter and thirteen centimeters in height. The heated ascitic fluid, in similar tall tubes, was steamed for thirty minutes in the Arnold sterilizer after the addition of sterile kidney. The tall tubes were also employed for ascitic-dextrose broth, ascitic broth, ascitic-dextrose-broth agar and ascitic-broth agar. To these were added sterile tissue and mineral oil, or one or both might be omitted.

Test-tubes taking a column of medium 1.5 centimeters in diameter and nine centimeters high were also employed for ascitic-dextrose broth, ascitic broth, and ascitic-dextrose agar. These will be called short tubes. Sterile tissue and oil might be added, but the oil was usually omitted. Six-ounce nursing-bottles, containing one hundred and fifty cubic centimeters of fluid in a column 4.5 centimeters wide and 8.75 high, were used for ascitic-dextrose broth or ascitic broth, with or without sterile tissue and always without oil.

To obtain the initial growth three media were regularly used: (1) Tall tubes of unheated ascitic fluid plus sterile tissue and oil; (2) ascitic-dextrose broth in (a) tall tubes plus tissue and oil, (b) short tubes with or without sterile tissue, and (c) bottles with or without tissue; and (3) ascitic-dextrose agar, usually without tissue. Forty to fifty tubes and bottles, with controls, were inoculated with central

nervous tissue at each autopsy. Sub-cultures were made to any of the media, and all tubes were frequently controlled by plating; further sub-cultures were often made from single colonies on the plates. The cultures were incubated at 35° C.; only a few were put in the anerobic jar, and it was thought desirable to make daily smears of all cultures which grew.

Cultural results. — A streptococcus identical to the one obtained from poliomyelitis in man was isolated from the central nervous system of each of the ten monkeys. Three other monkeys which did not have poliomyelitis gave completely negative cultural results. The number of pure cultures obtained from a monkey varied considerably through the series. Approximately fifty per cent of the cultures were sterile; of the remainder about fifteen per cent showed contaminations only, fifteen per cent showed the characteristic diplococcus and also contaminations, while the remaining twenty per cent contained the streptococcus in pure culture. Tubes inoculated with fragments or emulsion of cerebrum were usually pure cultures of streptococcus or sterile as this was the tissue which could be obtained with the least risk of contamination by air bacilli.

Cultures in similar media of blood or spinal fluid; and of emulsions of heart muscle, liver, spleen, and kidney were always sterile. In a few instances green-producing streptococci were isolated from lymph glands.

In aërobic bottles or short tubes of broth, with or without tissue, a diffuse turbidity appeared in twenty-four to forty-eight hours. Those inoculated with emulsion grew more often than those inoculated with fragments. Smears at this time (Fig. 2) showed elongated diplococci, often in short chains and occasionally in long chains. Giemsa stain frequently brought out a narrow clear zone about the organism which suggested a capsule; in a few instances this zone took a definitely red color. Sub-cultures on blood-agar plates invariably gave a growth of small, dry, green-producing colonies, which might be surrounded by a narrow hazy zone of hemolysis, especially after forty-eight to seventy-two hours

incubation. In shake cultures of the emulsion in short tubes of ascitic-dextrose agar small, discrete, grayish colonies appeared in the deeper portion of the medium, usually on the second or third day.

As was pointed out by Flexner and Noguchi,[2] the best medium in which to obtain the small globoid form was unheated ascitic fluid in a tall column to which fresh sterile rabbit kidney and oil were added. These cultures grew better when inoculated with fragments of nervous tissue rather than emulsions. Similar tall tubes of ascitic-dextrose and ascitic broth plus tissue and oil showed growth less frequently, and the organism was more apt to die after a few days.

We will now consider the growth obtained in the initial cultures of ascitic fluid plus sterile tissue and oil. All tubes were examined twenty-four, forty-eight, and seventy-two hours after inoculation: if any organisms were found in the smears, those tubes were examined daily thereafter. With a long fine pipette a small amount of medium was drawn from near the bottom of the tube; and two or three smears were made. As a routine, two of these were stained by the rapid and slow Giemsa methods described by Flexner and Noguchi;[2] and sometimes a third was stained by Gram's method. From the same pipetted specimen sub-cultures were made on blood-agar plates and other media.

In all tubes which did not remain permanently sterile, organisms were demonstrated in the smears made during the first forty-eight hours after inoculation; usually on the first day, but occasionally not until the second. These organisms were always similar to those found at the same time in aërobic broth cultures of the same material. They were usually elongated diplococci, occurring as pairs and short chains (Figs. 5a and 6a), or in long chains (Fig. 3a). There was almost always a clear zone around the diplococci suggesting a capsule; in a few instances the Giemsa method stained a capsule, making the diplococci appear larger and somewhat irregular (Fig. 4). In these smears there were frequently

cocci, sometimes singly, but often in chains, which showed early transverse fission, giving as a result a diplococcus resembling a meningococcus with flat sides opposed (Figs. 7c and 7d). The ascitic fluid medium was at this time absolutely clear throughout.

After the day on which the initial growth was first found there was a wide limit of variation in the type of growth. The tendency was for the organisms to grow smaller by a process of fission. In some cases the change was slow; sometimes it was three or even four weeks before the microorganisms were all of globoid size (*i.e.*, .15 to .3 millimeter). Figures 5a, 5b, and 5c give an example of such a tube; in these instances the medium began to grow hazy about the tissue on the sixth to eighth day; the slight cloud rose slowly through the lower third or half of the column, reaching its height about the end of the second week.

More frequently the time required for a culture to develop to globoid size was from five to ten days. Such tubes were hazy at the bottom about the fifth to the seventh day. The mechanism of the change in all tubes which showed a pure culture of small forms in six or more days appeared to be one or more transverse divisions of the individual cocci in the chains. Tubes in which a haze appeared earlier — on the third or fourth day — exhibited a much more rapid decrease in the size of the organisms, and rarely showed a pure culture of globoid size. The individual cocci divided transversely and then longitudinally, so that one large diplococcus broke into four small diplococci (Fig. 3c). The resulting cocci have been seen in process of dividing transversely once more, but this could not be photographed. In such a tube, on the second or third day every size might be seen in the same field — lanceolate diplococci, flat-sided diplococci, small cocci in clumps of four or eight, and very small globoid-size diplococci. Rarely such a tube showed pure globoid bodies on the fourth to sixth day, but usually larger organisms were present. A second cycle has been observed in these tubes: larger forms gradually appeared after the fourth to the eighth day up to about the fourteenth day, when these began to

divide and grow smaller again, becoming entirely small forms about the fifth week. However, this was an infrequent occurrence, and it was the rule for such tubes to show both large and small forms indefinitely.

Occasionally the capsule of the organism took the Giemsa stain deeply enough to obscure the size of the diplococci. When this occurred it was true with both the slow and rapid Giemsa methods, except in one instance. Throughout this series, of which examples from the first to the fourteenth day are shown, the smears stained by the rapid method showed a faint zone about the diplococci, while the smears made from the same pipette and stained by the slow method showed a capsule which held the stain strongly (Fig. 6a to f).

Sub-cultures were made on blood-agar plates at frequent intervals. As long as there were large organisms in the tubes, a growth of small green-producing colonies occurred on the plates. When only small globoid forms were present there was no growth on the plate. Stab cultures in tall tubes of ascitic fluid agar plus tissue and oil, showed a tendency to grow well up to the top of the medium when large forms were present, and to stop growing at an increasingly greater distance from the top as large forms became scarce and small forms more numerous. Sub-cultures from single colonies on blood-agar plates grew small in ascitic fluid plus tissue and oil, as did similar sub-cultures from aërobic broth tubes. By transferring a rather large inoculation from any type of initial culture to ascitic fluid plus tissue and oil, it was possible to cause a rapid breaking up of the organisms, with cloudiness of the lower portion of the medium which began in twenty-four to thirty-six hours, and soon extended to the top. Such transplants were especially favorable for studying the mechanism of fission, for in a smear of a twenty-four-hour culture all stages could be demonstrated (Fig. 7a to d).

Cultures in ascitic fluid plus tissue and oil which showed only globoid bodies, and which would not grow in aërobic broth or on a plate, were grown back to large lanceolate diplococci without difficulty if they were not more than

three or four weeks old. Those which had been under comparatively anerobic condition for a longer time grew large more slowly. It was found that tall tubes of ascitic fluid plus tissue and oil which had been steamed in the Arnold sterilizer gave a favorable medium for the first transplant of the globoid bodies. Transfer to short tubes of ascitic-dextrose broth plus tissue was often a successful second step. Another good method to obtain the large aërobic forms was to stab tall tubes of ascitic-dextrose agar plus tissue, and to make sub-cultures from the highest point of growth.

Cultures and emulsions of brain and cord of paralyzed monkeys were passed through Berkefeld N filters; a small percentage of these grew in unheated ascitic fluid plus tissue and oil, but not in other media. By methods similar to those described above culture of the filtrates were grown up to the large form.

It was noticed that in tubes which were contaminated with B. subtilis, the diplococci became small rapidly, and tended to remain small. Sub-cultures of several strains were inoculated with B. subtilis. Figures 8a and b show the typical result of such experiments. In the nine-day smears the contaminated tube showed only globoid chains, while the uncontaminated control showed the pleomorphic organism in different stages of division.

The tendency to break down to small forms under proper cultural conditions was not peculiar to the diplococcus isolated from poliomyelitis material. Strains of pneumococci and streptococci from various sources have been planted in ascitic fluid plus tissue and oil; some of them have gone through precisely the same changes, and in two instances they have been passed through a Berkefeld N filter and grown large again. Figure 9 shows various stages of division with resulting globoid-size organisms in a culture of a five-year old pneumococcus, isolated from the blood during life in lobar pneumonia and which had passed through thirty-five animals. This strain had always killed the animals by pneumococcemia. It had been in dried

mouse spleen in a sealed tube in the dark for three years at room temperature. Before inoculation of the tube from which a smear is shown, it had been sub-cultured twice from single, large, moist, green colonies on blood-agar plates.

Discussion. — On examining again preparations made of cultures from the central nervous system of cases of human poliomyelitis, it is now possible to place the various forms — lanceolate diplococci, rounder cocci, and especially the "rather large oval cocci staining a pinkish tint with the Giemsa stain" — which we described in the previous report.[1] Flexner and Noguchi[2] noted in old cultures "enlarged and irregularly-stained bodies" which they considered were probably degenerations. They also described a faintly-tinted or colorless zone which sometimes surrounded the globoid organisms.

The diplococcus isolated from the central nervous system and intervertebral ganglia of ten monkeys which were given experimental poliomyelitis by means of an adapted virus, remains an ordinary-sized diplococcus under aërobic conditions. Sometimes a capsule is demonstrable. Under conditions of low oxygen tension the same organism may appear in twenty-four or forty-eight hour cultures in ascitic fluid plus tissue and oil. Many factors appear to play a part in determining the type of growth after this. With a suitable specimen of ascitic fluid and fresh sterile tissue, and with a scant initial growth, the organisms become small slowly by transverse fission. In such tubes there may be a pure growth of globoid organisms after five to fifteen days. The medium is clear while the large forms predominate. With similar medium and larger initial growth, especially in sub-cultures, the fission is first transverse and then longitudinal, and the resulting cocci go through further division. Such tubes become turbid early — second to fourth day — and all sizes of the organism may be found in early smears. These tubes seldom become pure cultures of globoid bodies, but they may go through a second cycle of division and

become pure small forms. Sometimes there is a demonstrable capsule in all stages of division.

If the ascitic fluid is not suitable, if the tissue is not fresh — or if fluid and tissue are steamed — division takes place slowly, if at all, and pure globoid cultures are not obtained. When such tubes are inoculated with B. subtilis, conditions are sufficiently changed to cause fairly rapid division, with resulting pure globoid cultures in some instances.

It seems possible that among many factors concerned in causing these changes in size, surface tension may be of importance. If there are so many large diplococci that their metabolic requirements cannot be met by the medium, division which gives more surface for the same-sized body might allow a certain number of the resulting smaller organisms to survive.

Under exactly right conditions of medium, tissue, and size of inoculation, it is conceivable that nothing but small globoid organisms would be found as early as the third or fourth day. Occasionally such a tube does occur, but it has always contained large forms on the first or second day, or on both. If each tube is not examined daily for three days, this stage may be overlooked. Such tubes do not show any clouding of the medium until the globoid forms are well established on the fourth to sixth day. Cultures which have been under anerobic conditions for some time are much more liable to grow in this fashion than are initial cultures; but in all of them forms well above globoid size may be seen in smears made on the first or second day.

This method of growth in accordance with the oxygen tension of the medium is not peculiar to the diplococcus isolated from poliomyelitis nervous tissue, but may be demonstrated, even to growth after filtration, by the use of streptococci and pneumococci from various sources. It therefore appears that similar methods may give valuable information if applied to the study of other diseases caused by a filterable organism or virus.

CONCLUSION.

The small globoid microörganism which Flexner, Noguchi, and their co-workers have considered to be the cause of experimental poliomyelitis has always, in our experience, been the result of the breaking down of large diplococci, which have been isolated from the central nervous tissues of each monkey infected with experimental poliomyelitis. These organisms have not been isolated from other tissues except lymph glands of poliomyelitic monkeys, nor from any tissue of normal monkeys. The mechanism by which the large forms become small has been demonstrated.

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DESCRIPTION OF PLATES VII.—XI.

Plate VII., Fig. 2. — Twenty-four-hour culture in ascitic-dextrose broth bottle plus emulsion of lumbar cord. Rapid Giemsa. x 1000.

Fig. 3. — Ascitic fluid plus tissue and oil, inoculated with fragment of cervical cord. To show breaking down by division in two planes.

a, Twenty-four-hour smear. Rapid Giemsa. x 1000.

b, Forty-eight-hour smear. Slow Giemsa. x 1000.

c, Forty-eight-hour smear. Chain of large diplococci; transverse and longitudinal division of one diplococcus into four pairs of diplococci, and clumps of the results of similar division. Slow Giemsa. x 1000.

Plate VIII., Fig. 4. — Ascitic fluid plus tissue and oil; first sub-culture from similar tube inoculated with fragment of cerebrum. Twenty-four hours. Excapsulated lanceolate diplococci, some of which have divided transversely. Rapid Giemsa. x 1000.

Fig. 5. — Ascitic fluid plus tissue and oil, inoculated with fragment of brain.

a, Twenty-four hours. Note transverse division of large diplococci forming two small diplococci of same morphology. Rapid Giemsa. x 1000.

b, Six days. Small diplococci, with suggestion of capsule. Rapid Giemsa. x 1000.

c, Twenty days. Globoid diplococcus and short chain. Rapid Giemsa. x 1000.

Plate IX., Fig. 6. — Ascitic-tissue fluid inoculated with fragment of brain. To show slow diminution in size; and staining of capsule by slow Giemsa method, while smears from the same pipette stained by rapid method do not show a distinct capsule.

a, Twenty-four hours. Rapid Giemsa. x 1000.
b, Twenty-four hours. Slow Giemsa. x 1000.
c, Four days. Rapid Giemsa. x 1000.
d, Four days. Slow Giemsa. x 1000.
e, Fourteen days. Rapid Giemsa. x 1000.
f, Fourteen days. Slow Giemsa. x 1000.

Plate X., Fig. 7. — Ascitic-tissue fluid sub-culture from similar tube inoculated with fragment of cervical cord. Twenty-four-hour rapid Giemsa smears.

a, Stage in breaking up of a diplococcus. The upper pair are result of transverse division; the lower pair have also undergone longitudinal division, and the lowest resulting diplococcus has swung 90° from its neighbor. x 1000.

b, Double fission of two diplococci into eight diplococci. x 1000.

c, To show all stages: lanceolate diplococci undergoing transverse fission into flat-sided diplococci. Further longitudinal fission and resulting small forms. Clear spaces in chains which contain small irregular reddish masses. x 1000.

Fig. 8. — Two tubes. Ascitic-tissue fluid sub-cultures of lumbar cord to show effect of B. subtilis contamination. Rapid Giemsa stain.

a, Nine days. Globoid chains and B. subtilis. x 1000.
b, Nine days. Stages of division in uncontaminated tube. x 1000.

Fig. 9. — Rapid Giemsa stain of heated ascitic-tissue fluid culture twenty-three days old of a five-year old pneumococcus strain. Compare with Figs. 3b and 7d. x 1000.

Plate XI., *d*, Camera lucida drawing to show further details of *c*.

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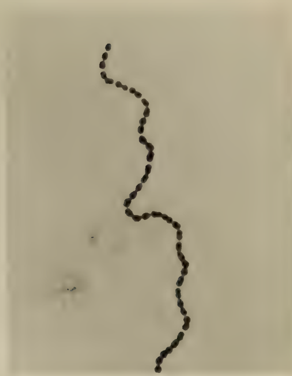

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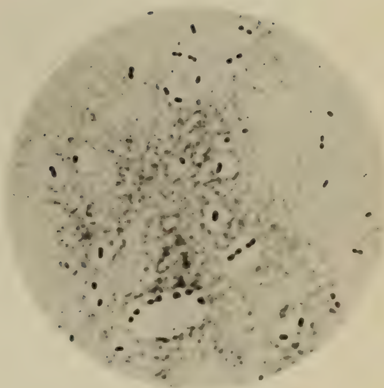

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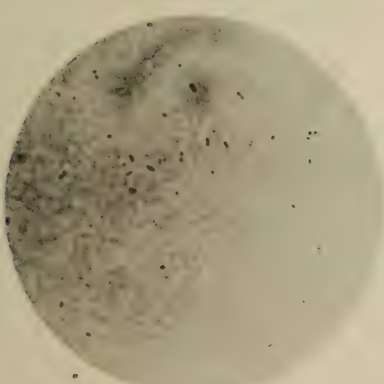

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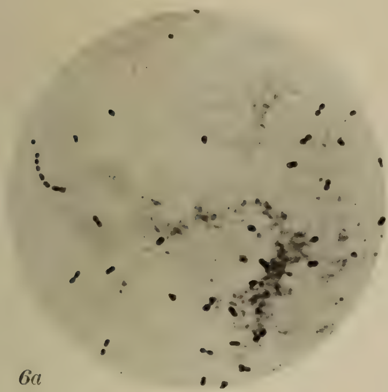

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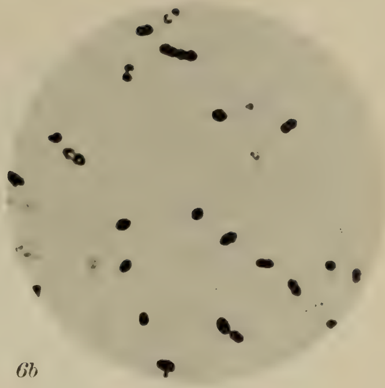

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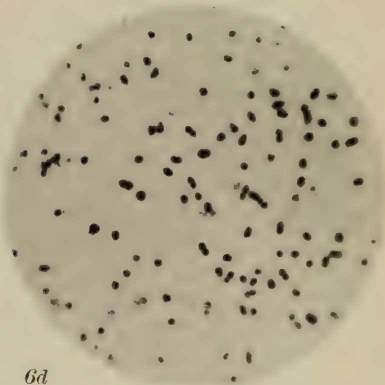

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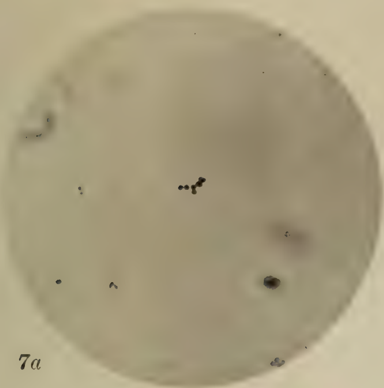

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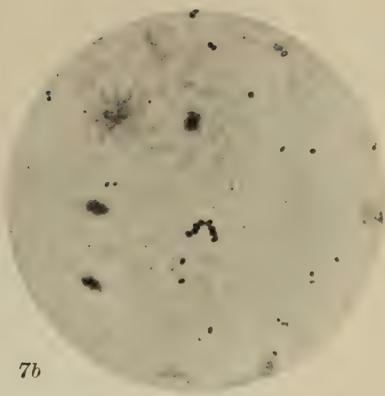

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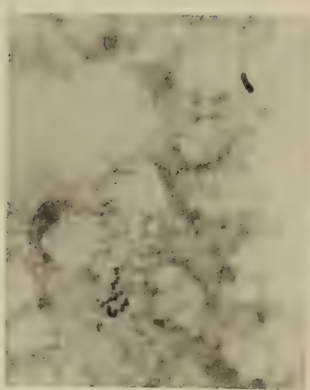

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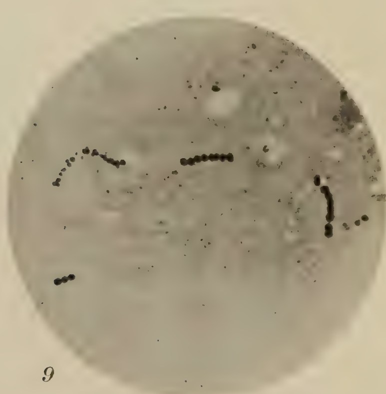

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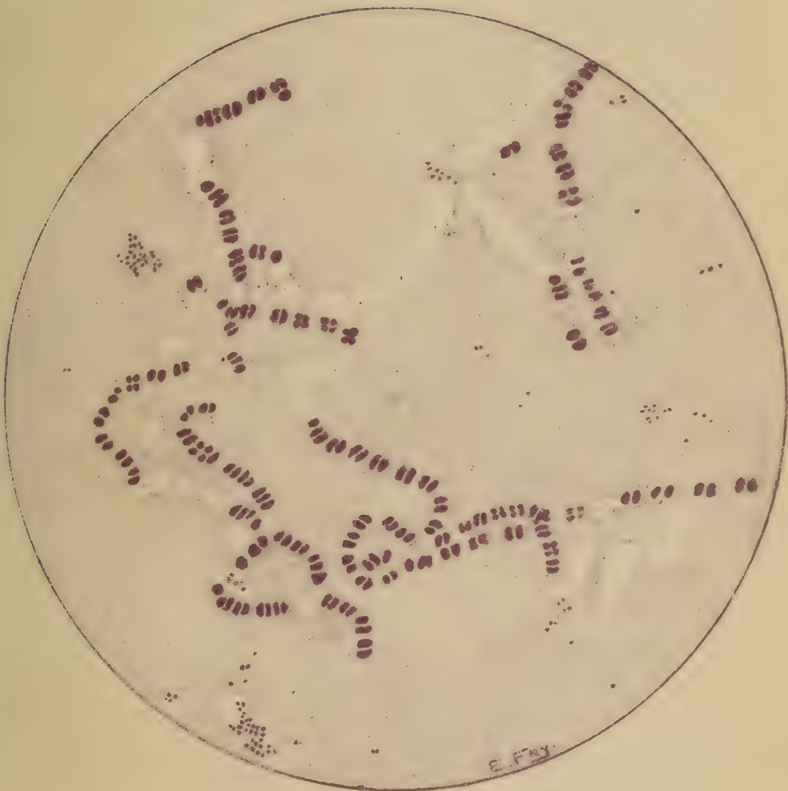

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Arthritis Deformans as an Infectious Disease.*

AN EXPERIMENTAL AND CLINICAL STUDY FROM THE CARNEGIE LABORATORY (UNIVERSITY AND BELLEVUE MEDICAL COLLEGE) AND THE MONTEFIORE HOME AND HOSPITAL FOR CHRONIC DISEASES.

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There is still far from unanimity in the interpretation of the pathological findings in chronic joint diseases. The majority of workers admit that the post-mortem findings cannot be brought in conformity with definite clinical conditions, and few are agreed as to the specificity of the various morbid conditions to be found at autopsy.

Some years ago I proposed to divide all these conditions into two primary divisions: viz., the inflammations, or infectious diseases, and the degenerations. However, as my opportunities for study have increased I have been forced to admit that this classification, though useful as a convenient basis for study and description, is more or less arbitrary. I have, like all others engaged in the study of these conditions, been forced to the conclusion that the various anatomical, particularly the histological abnormalities, although characteristic in themselves cannot be brought in correspondence with specific etiological factors, clinical entities, or even definite pathological entities.

This is true more particularly of the polyarticular joint conditions. In these the pathological processes in the

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various joints often present dissimilar or opposite conditions. So there may be purely atrophic and purely proliferative conditions or combinations of the two in the joints of the same individual, and though there may be only synovial changes in some, and only osseous changes in others, combinations of such changes are often to be found side by side in the different joints of the same individual.

Even in the cases of known etiology the search for a specific reaction in the joint tissues in correspondence with specific morbid factors has been in vain. In tuberculosis and lues, though we are able to demonstrate the specific changes which these organisms induce in all tissue, the reactive processes in the joint structures are fundamentally the same as those induced by other morbid influences. We may have a distinctly synovial type of tuberculosis with the formation of granulation tissue (pannus) with secondary invasion of the cartilage and bone; or we may have on the contrary a primary osseous focus which invades the cartilage and the joint from the epiphysis. The epiphyseal disease may present purely degenerative, or purely inflammatory changes, or a combination of the two at autopsy. These changes may be accompanied by a proliferative process in the joint interior resulting in fibrous or bony ankylosis, or a focus may remain within the epiphysis causing atrophy of the neighboring bone structures, which subsequently leads to deformation without involving the articular cartilage.

Exactly analogous phenomena occur in gonococcus, pneumococcus, and other infections. The differences are simply in the extent and the rapidity of the process and not in the fundamental changes in the joint tissues.

Similar conditions also prevail in the monarticular forms of so-called osteo-arthritis deformans, particularly in the hip joint. In some cases there is definite subchondral atrophy and marked deformation of the head of the femur without demonstrable histological change in the joint cartilage or the capsule; in others, the cartilage is entirely degenerated, the capsular changes are marked, and there are proliferative, chondral, and osseous changes with or without concomitant

bone atrophy. Between these two extremes there are all grades of transition.

Hence, as both the proliferative and the degenerative phenomena occur in the joints of the same individual in polyarthritis, and side by side in these, as well as in cases of monarticular disease, both in those forms of known etiology, such as tuberculous, gonococcus, and pneumococcus infections, and those of unknown origin, it must be evident that these morbid processes are unsuitable as a basis for the classification of the joint diseases.

It is therefore impossible, without leading to confusion, to divide the joint diseases according to these pathological findings. There are no joint diseases which are exclusively degenerative or exclusively proliferative; the one or the other may predominate in a particular joint, but there are evidences of both these conditions in practically all joint diseases.

The reason for this becomes evident when these phenomena are studied, not only in their connection with joint diseases, but in their relation to analogous pathological phenomena as they occur elsewhere.

Vascular changes, round cell infiltration, and the formation of granulation tissue, the changes said to be characteristic of proliferative forms of joint diseases, are simply the phenomena of inflammation. These morbid processes may be followed by partial or complete resolution, or they may be followed by degenerative changes. Thus depending upon the stage, the intensity, or the duration of the disease, joints, like other organs the seat of inflammatory conditions, no matter what the cause, may show any one of or all these changes. Thus active inflammatory and degenerative changes may be present side by side in the same joint. Or, when the inflammatory condition has long subsided, there may be simply degenerative changes with nothing to show that these changes were preceded by a more or less intense inflammatory condition.

I have been able to demonstrate that such degenerative changes may be preceded by intense inflammatory conditions

in a number of cases. Figure 1 is a section of the juxta-articular portion of the femur from a boy who has been in my care for the past eight years. This patient, who is now twenty-two years old, has had what appeared to be a recurrent general infection in which a considerable number of the joints were involved. He recovered with ankylosis of both hips and knees, and considerable deformation of the hands and wrists. In order to permit him to sit up to read and write (he is both intelligent and ambitious) among other things I resected both hips, producing a flail joint. The section here illustrated is from the right hip, which originally was the site of a very acute and very intense inflammatory condition, with marked effusion and complete dislocation and ankylosis.

As is shown in the illustration the subchondral region is composed almost entirely of fat. The few extremely atrophic bone trabeculæ which still remain are, upon microscopical examination, seen to be composed of minute spicules of dead bone. Immediately beneath the joint cartilage there are seen subchondral bone cysts which in no way differ from those described by Ziegler as characteristic of the monarticular forms of so-called arthritis deformans, which I with many other writers formerly believed to be typical of a degenerative condition. Microscopically, too, the findings differ in no way from these conditions. The joint cartilage is covered with a thin layer of non-vascular connective tissue and is degenerated. The subchondral bone trabeculæ have for the most part disappeared and the greatly enlarged marrow spaces are almost entirely filled with fat. However, in spite of the widespread and apparently typical degenerative process, there are still areas, minute it is true, which indicate that the process was preceded by an inflammation. Thus, as shown in Figure 2, there are distinct areas of granulation tissue still to be seen upon what was the joint surface, and within the marrow there is still evidence of the proliferative phenomena which, as will be shown later, usually accompany inflammatory bone conditions.

It can be argued that the insignificant inflammatory areas

in the midst of a widespread degenerative condition are secondary — due to the irritation of function under abnormal conditions; but, in this particular case, the hip was immovable and the patient was in bed; so that secondary irritation, even were the previous condition unknown, could be excluded. It is known in this case that the degenerative phenomena were preceded by marked signs of inflammation, so there can be no doubt that the inflammation was the primary and the degenerative condition an end result.

Such examples could be multiplied; and as there is rarely an instance of a degenerative joint condition in which some signs of a concomitant or preceding inflammation are not demonstrable, it is evident that a degenerative condition is in many cases simply the terminal stage of an inflammatory process.

The possible existence of a primary degenerative process in the joint structures cannot of course be denied absolutely. The presence of such changes in long standing cases of joint disease does not, however, prove that the process is primarily degenerative in character. It is useless, therefore, as I have pointed out before, to draw conclusions from specimens removed from aged persons, particularly in those cases in which the duration or the clinical history of the disease are unknown. Degenerative changes in such cases may be, indeed nearly always are, simply the terminal stage of some inflammatory process. They are found not only in the joint structures in diseases of obscure origin, but also in those of a frankly infectious or inflammatory nature, such as tuberculosis and gonorrhea. Hence, though it is conceivable that primary degenerative conditions affect the joint structures, it must be concluded that in the majority of cases such changes are secondary to inflammatory conditions. Here, as elsewhere, degenerative conditions, when they follow inflammations, denote the more or less complete inhibition of the reparative process after the active morbid condition has subsided.

Moreover, the variation in the reparative tendency following inflammatory conditions, its complete inhibition, partial,

complete, or superadequacy, readily accounts for many of the variations in the pathological findings of the terminal stages of joint diseases. In all inflammatory conditions the tissues which are destroyed are, when the process goes to completion, absorbed and subsequently regenerated or replaced by connective tissue. When the condition is active and the terminal reparative processes occupy a short space of time, it is said to be acute; when, on the other hand, the phenomenon of invasion, destruction, and reparation occupies a long time, or when the reparative processes are incomplete, the condition is said to be chronic.

So, too, in joint disease we have acute conditions which run their course rapidly. We have, on the other hand, those in which the termination is either remote or entirely indeterminate. In either case the process may end in complete resolution; the reparative activities may be absent or incomplete, or they may be superadequate, but ineffectual in restoring the morphological correspondence to normal function.

For instance, in the case just cited, an acute inflammatory condition terminated in the stage of degeneration; the degenerated tissues were not absorbed, and the reparative processes remained in abeyance. Here we find, when the tissues are examined, what appears to be a typical degenerative condition. Again, the absorption of the necrotic material may be more or less complete, but not followed by repair; we then have simple atrophy of the subchondral structures as a terminal stage, and it is more than likely that, as will be shown later, the so-called atrophic joint diseases are typical of such a termination.

On the other hand, there are instances in which the reparative processes, though present, are incomplete. So, for instance, when defects in the cartilage are permanent or replaced by connective tissue, the normal contour of the joint surface (destroyed during the stage of softening) is not restored. Thus, as a result of an acute or a chronic condition (for the duration or the activity of the process makes no essential difference in the changes), we have a terminal stage

of incomplete resolution. In such cases the mechanical interference with joint function will continue to exist, though the actual pathological process has terminated.

In contrast to these conditions are those in which, like inflammations elsewhere, the active stage of invasion, degeneration, and absorption of necrotic tissue is followed by excessive reparative activity, viz., connective tissue proliferation in the subchondral narrow spaces, the joint interior, and the capsule. Such proliferative phenomena may be due to the organization of the granulation tissue or the invasion of connective tissue from surrounding areas; or the fixed tissue cells may take part in the proliferative process. In this connection it is important to consider the embryonic origin of the joint structures, for when these are understood, the interpretation of many of the phenomena of joint disease, otherwise inexplicable, becomes a matter of little difficulty.

The original skeletal elements are laid down in cartilagenous anlages within the scleroblastema derived from the mesenchymal cells. The cartilagenous anlages gradually approach one another, and apparently have a tendency to coalesce. But at a time when they are almost in contact dehiscence takes place in the scleroblastema and thus is formed the joint cleft. At this stage the cartilagenous skeletal elements are surrounded on all sides by a layer of condensed scleroblastema. The scleroblastema cells within the cleft subsequently disappear and those which remain in contact with the cartilage peripherically become modified to form the perichondrium. The perichondrium does not undergo segmentation, but remains continuous, binding the skeletal elements together and subsequently forms the joint capsule. Later, when ossification takes place, the cells which form the internal layer of the perichondrium undergo modification of some kind so that they produce bone instead of cartilage, those of the capsule change their shape, but only to a certain extent their biological characteristics, to form the synovial lining to the capsule.

The histological details do not concern us at the present

time. What is important, as bearing upon this subject, is the fact that the cells of the periosteum, the perichondrium, and the joint capsule are derivatives of the same embryonic cells. And though the majority of the cells in the periosteum, the perichondrium, and the capsule have been differentiated, so that they develop bone, cartilage, or synovial cells in these regions in adult life, a considerable number of these cells undoubtedly retain their embryonic potentialities. Thus real cartilage cells and cells which are transitional between the cartilage and the synovial cells are not uncommon in the normal capsule. In the region of the capsule insertion, cartilage, synovial, and transitional cells are numerous. And though the cells in the cambium layer of the periosteum seem to have reached a stage of differentiation which renders them incapable of forming anything but bone cells under ordinary circumstances, they apparently revert to embryonic conditions upon the advent of unusual stimulation. Thus these cells produce cartilage cells as well as ostoid cells in the repair of fractures.

The cells in the capsule, in the periosteum, and in the area of transition between the capsule cartilage and periosteum will, under morbid influences, proliferate and produce bone or cartilage in the regions where these tissues are found under normal conditions. Under some circumstances apparently the cells proliferate as embryonic cells and finally produce bone or cartilage in situations where these structures are absent under normal conditions. Thus large masses of cartilage may develop in the capsule; enchondrosis or osteophytes in the "capsule cartilage periosteum transitional area;" and cartilage beneath the periosteum or within the medullary cavity.

It is therefore unnecessary to assume, as many pathologists have done, that cartilage or bone in abnormal situations within the articular structures is the result of metaplastic process. Indeed this assumption is evidently unjustifiable upon a priori grounds. It is well known that specific bone and cartilage cells have reached a stage of differentiation which renders them incapable of propagation, or any change

except death. These cells have no power to replace themselves, and the regeneration of bone or cartilage takes place by means of the intermediate cells in the marrow, the perichondrium, or the periosteum. And, as has been explained, the latter to a certain extent retain their embryonic character, hence it should not be a matter of surprise that under certain circumstances, or certain mechanical conditions which cannot be dwelt upon at the present time, bone is formed instead of cartilage, or vice versa. That these structures are formed in excess is simply due to the influence of the unusual stimulation.

Thus the presence of the various hypertrophic processes which occur in the course of or as a result of joint disease are readily accounted for. It is unnecessary to assume that these conditions are metaplastic or that they are due to specific morbid influences; their presence undoubtedly depends upon the location, the intensity, and the duration of any irritative pathological condition and its effect upon the mechanical function of the joint structure.

In the exposition here given it has been necessary for spatial reasons to avoid all the details of the conditions discussed. However, as the histological details and the pathology of the chronic joint diseases have been accurately and fully discussed in the admirable paper on this subject by Nichols and Richardson, a detailed description is unnecessary.

From what has been said it must be concluded that, with the exception of the specific changes which certain microorganisms induce wherever they become localized (for instance the tubercle bacilli and spirochetes) all the morbid changes to be found in joint disease can be ascribed to variations of an inflammatory process. The changes are not specific; the variations are not reactions to definite etiological influences; nor do they correspond to definite clinical entities.

The variations are due to the variations in the location, the intensity, the duration, the termination of the process and stage in which the affected structures are examined.

The hypertrophic and the so-called metaplastic processes

are due to the reaction of the quasi embryonic cells in the joint structures: their presence or absence, their intensity and duration, may be readily accounted for by the localization, duration, and intensity of morbid influences combined with alterations of the mechanical conditions within the joint structures.

Though there was no doubt in my own mind that these conclusions were correct and could be verified by any one with the pathological material at his disposal, it seemed to me desirable to verify the findings and their interpretations by reproducing them experimentally. The more so as it seemed to be possible, if such experiments were successful, to acquire in this way a more definite knowledge of pathogenesis of chronic arthritis.

That it is easy to induce acute or suppurative bone and joint lesions experimentally in animals is a fact which was well established some time ago. But all attempts to induce chronic non-suppurative conditions were unsuccessful until J. Koch succeeded in producing such conditions by inoculating dogs with hemolytic streptococci.

J. Koch's first experiments were performed with the intention of determining the localization of microörganisms in the bone marrow. Before him Lexor had shown experimentally, and by studying the vascular conditions in the bones by means of radiography of injected bones, that bacteria become localized in the bones by infarction in the terminal capillary circulation, and that for this reason the regions in which these terminal capillaries are situated are those in which bacterial infections originate.

Koch's experiments confirm the work of Lexor. He inoculated young rabbits with anthrax, streptococci, and pneumococci and found that the principal sites for the localization of the microörganisms are (1) the endosteal region of the primary medullary spaces in the line of ossification; *i.e.*, at the point where the capillaries abut the growing cartilage, within the column of calcified cartilage. (2) The large venous channels in the epiphyseal marrow. (3) The terminal subperiosteal vascular region.

The bacteria soon injure the vessel wall and then escape to the cartilage or marrow spaces. The veins particularly are seriously damaged; they become congested and dilated, subsequently contain thrombi, and the bacteria escape to the marrow spaces. In the subperiosteal region the bacteria follow the vessels which run parallel with the long axis of the bone into the neighboring bone structures, where they form first infarcts, and then foci of infections.

In the rabbit Koch was able to induce acute, sub-acute, and sub-chronic streptococcus infections with suppurative articular lesions. He found it more difficult to demonstrate the streptococci while they are still within the endosteal vessels, though the fact that these show pathological changes (congestion, thrombosis, and fibrin content) plainly indicate that the vein must have been their original point of localization. The organisms are found in isolated, irregularly-distributed foci in the region of the terminal endosteal vascular system. Another point of localization, one which has a particular bearing upon the pathology of human joint disease, is the vascular region beneath the epiphyseal periosteum and the perichondrium. Though the other microörganisms studied also frequent this locality it seems to be a point of predilection for the streptococcus (erysipelatis). Here the latter form large foci which are not, as are those of other microörganisms, confined to the perivascular regions, but, just as they do in the skin, the streptococci invade the lymph and other tissue channels, causing widespread inflammatory changes.

The invasion of the meta-epiphyseal region of the endosteum and subperiosteum is at first focal; but depending upon the virulence of the organism or the susceptibility of the animal, the inflammatory changes which ensue soon spread more or less rapidly to the neighboring spaces. The earlier changes within the marrow spaces are hyperemia, capillary hemorrhage, and proliferation of the marrow cells. Soon new-formed vessels make their appearance, and within a short time approach and finally invade the columns of calcified cartilage. The cartilage cells at first proliferate, but

they are soon absorbed and the epiphyseal line becomes irregular, very strikingly simulating the epiphyseal cartilage changes in human rachitis.

The microörganisms disappear from the blood very soon after they are injected; nor can they be recovered from the joint exudate which usually ensued, though the organisms could be demonstrated in the epiphyseal marrow both by culture and microscopical examination.

Koch then began experimenting upon puppies. By injecting the streptococcus longus (erysipelatis) he was able to induce a condition which rather closely resembles the condition usually designated as acute articular rheumatism in the human subject. Following a period of incubation varying from one to three days, there were signs of general indisposition, an insignificant rise in temperature, joint effusion, and diarrhea.

The severity and duration of the general condition varied considerably. In some dogs the general indisposition was insignificant, the joint symptoms evanescent, and the animal was apparently well in a few days. In some the general reaction continued only a few days, but joint swelling continued for ten days or more. In a few cases the joint condition was progressive, the joint exudate assumed a purulent character containing enormous numbers of streptococci, and the animals died of secondary sepsis (general infection). In some instances there remained localized periosteal thickening after the intra- and peri-articular swelling had subsided. The serous or fibro-serous exudate involved not only the joint, but the peri- and para-articular structures; and in the severe cases the para-articular tissues become so distended by the edema that they remained dilated when the fluid was finally absorbed.

The exudate contains numerous polynuclear leucocytes, but in the majority of the cases no bacteria. In such cases, though the intra- and peri-articular exudate remains sterile, the microörganisms could frequently be recovered from the adjacent bone marrow.

The general infection rapidly subsides. Repeated blood

cultures are negative even during the stage of active joint disease when the microörganisms may be recovered from juxta-articular bone marrow.

The pathological changes are similar to those found in the rabbit and for the most part involve the juxta-epiphyseal region of the epiphysis, the inner layer of the periosteum and perichondrium. In a few instances Koch was able to follow the morbid process to the time when the joint cartilage was about to be invaded by new-formed blood vessels and marrow tissues.

In my first series of experiments twelve dogs were injected with a hemolytic streptococcus furnished by Dr. Noble of the bacteriological department of the Carnegie Laboratory. (Because the clinical and technical details are identical to those described by Koch, the protocols are omitted in order to save space.) Two of the animals died maurantic after twenty and twenty-eight days respectively. The others were killed at intervals from the third to the ninetieth day. All the large joints with the epiphyseal ends of the bones, and the spine, whether they showed macroscopical changes or not, were microscopically examined. In this way it was possible to obtain a series of specimens which showed all the pathological changes, from the earliest period of juxta-epiphyseal infection to more or less complete epiphyseal destruction.

Following a period of incubation of about three days practically all the animals of this series showed more or less definite symptoms of general indisposition. Only a few, however, had a marked rise in temperature, and this rise, whether high or moderate, except in two instances, continued for only a short time — three or four days.

The general condition varied; some of the animals were quite ill, refused to take food, and were apparently unable to stand; in others the general symptoms were very mild and soon subsided, and in a few of the cases the general indisposition continued for about ten days. Variable, too, was the

relation between the intensity of the general reaction and the joint symptoms.

Clinically the most characteristic and definite types of infection were the following: 1. Acute general reaction, acute joint symptoms, complete recovery. 2. Acute general reaction which subsides, chronic joint conditions which may eventually subside. 3. Acute general reaction, chronic and progressive joint lesions, with permanent impairment of joint function. 4. Acute evanescent general reaction, no joint reactions, or a mild joint reaction which rapidly disappears; subsequently there is progressive deformation of the limbs. 5. Animals in whom the general indisposition and the weakness of the limbs is progressive, leading, in two of my cases, to a maurantic condition and death.

As regards the early stages of epiphyseal involvement, my microscopic findings are in accord with those of Koch. Besides the marrow changes there is an intra- and para-articular joint exudate, and in this connection it is important to remember that Koch, who repeatedly examined this exudate bacteriologically, found it sterile. Though no bacteria could be demonstrated in the joint exudate in these particular cases, the streptococci could nearly always be demonstrated in the neighboring epiphysis; so that in the earlier stages of epiphyseal infection the joint exudate may be considered symptomatic. That such symptomatic joint exudates occur with other forms of infection was demonstrated by Lexor in his experiments with staphylococci. They are known to occur in juxta-articular, acute, and chronic osteomyelitis in the human subject. The fact that the joint effusion may be symptomatic no doubt accounts for the failure to recover the microörganisms from the joint fluid in many cases of joint infection in the human subject.

The analogy between the evanescent peri- and para-articular sterile exudate, and the conditions found in cases of so-called acute articular rheumatism and a certain percentage of the cases of so-called gonorrheal rheumatism is striking. Moreover, the mild, repeated joint swellings commonly found in all forms of infections — measles, scarlet fever, typhoid,

pneumonia, sepsis (general infection due to an unknown microörganism) — are also, no doubt, analogous. These arthritides are therefore probably not of toxic origin, but the synovitis is due to the bacteria in the adjoining epiphysis.

Another point very important because of its bearing upon human pathology is the fact that the joint disease may remain active and progressive though the symptoms of general infection have completely subsided. Thus the condition in the epiphysis may become purely local, unaccompanied by general indisposition and temperature. This was strikingly illustrated in several cases in which the local changes were marked and progressive, though the animals showed absolutely no signs of general illness. In the dogs of this series the inoculation was followed by a surprising diversity of pathological changes in the epiphyseal ends of the bones. As the dose of the microörganism injected was the same this diversity was no doubt due to the variation in susceptibility, the stage when the animal was examined, the location of the epiphyseal infection, and the age of the animal.

In all cases the earliest epiphyseal change to be seen microscopically is either local or general hyperemia. In the present paper only the changes which lead to or are likely to lead to joint involvement are to be considered. There may be one or a number of foci. The vessels are filled with blood, the veins are markedly dilated and become thrombosed as the condition proceeds. At a time, which no doubt corresponds with the period when the microörganisms escape from the blood vessels, the marrow cells in the neighborhood of the focus or throughout the epiphysis rapidly increase in number and soon more or less completely fill the marrow spaces. As the congestion and marrow cell hyperplasia increase, the bone trabeculæ are gradually encroached upon and begin to undergo absorption.

The giant cells are markedly increased in numbers and are irregularly distributed in the medullary spaces. These cells are not located near the bone trabeculæ, nor are there any large multinuclear cells which in any way resemble the cells

described as osteoclasts to be seen in considerable numbers anywhere. These cells are in fact the myeloblasts so often found in the granulation tissue associated with bone inflammations generally. In this case at any rate the bone absorption is evidently not due to specific cells at all; but the bone apparently disappears upon the approach of the new-formed vessels and proliferating marrow cells. Here, as elsewhere, the bone makes way for encroaching soft tissue, and it is apparently the change in the vascular conditions or the resulting increase in the intramedullary pressure which is responsible for the bone absorption.

The question which has so long excited controversy amongst those engaged in the study of bone pathology, viz.: whether the bone is at first decalcified and then absorbed, or, on the contrary, the calcified bone is absorbed and the softening, so often associated with pathological bone conditions, is due to the failure of the new-formed bone to become calcified, might be discussed in this connection. I do not, however, consider this question of fundamental importance in the present connection and even if answered would not throw much light upon the pathogenesis of the conditions now under discussion. It may, however, be well to state that the evidence one way or another to be obtained by experimental streptococcus infection is distinctly in favor of a condition of decalcification as the initial morbid process. There can be no doubt that following epiphyseal infection in dogs, the bones rapidly became softer. For under normal circumstances the dogs' bones are extremely hard and very difficult to decalcify and cut in thin sections for microscopical examination, whereas the bone from the affected epiphysis is very readily decalcified and sectioned. The softening cannot, in my opinion, be wholly due to the actual absorption in all cases, for it exists during the very early stages of the infection, before extensive absorption or new bone formation have supervened; and when the infection remains focal there is a considerable area of softening not actually invaded by the inflammatory process surrounding the focus.

Whether the softening is primary or secondary, it is

remarkable how soon after bacterial invasion the bone rarefication begins. In some cases the marrow spaces of entire epiphysis were considerably enlarged and filled with lymphoid marrow within three days following inoculation.

For the present only the juxta-articular infection is to be considered. The manner in which the juxta-articular focus invades the joint, other things being equal, depends upon its location. The focus may originate primarily within the marrow tissue, or as frequently happens, its original location may be in the sub-periosteal region of the epiphysis. Though foci are usually found in both locations simultaneously and though marrow changes nearly always accompany subperiosteal foci, the manner in which these two invade the interior of the joint will be considered separately in order to facilitate the description.

The earliest change in the vicinity of the epiphyseal focus is an intense hyperemia; soon numerous hemorrhages make their appearance; the marrow cells and giant cell proliferation proceeds rapidly, and the marrow spaces in the area involved rapidly become filled with intensely engorged blood vessels and lymphoid marrow containing numerous giant cells.

As the process advances the bone trabeculæ become absorbed, adjacent marrow spaces become confluent, and in the very severe cases the entire juxta-articular epiphysis becomes converted into lymphoid marrow, containing numerous giant cells, almost devoid of bone trabeculæ (Fig. 3).

The cell proliferation and bone absorption advance in all directions (when there is more than one focus these coalesce), and encroach upon the epiphyseal line on one side and the joint cartilage upon the other. When the inflammatory area approaches, but does not actually invade the chondral structures, there is some tendency (particularly in the epiphyseal cartilage) to irregular cartilage cell proliferation. The stage of cartilage proliferation is, however, limited in extent and of short duration. As the pathological process advances and actually encroaches upon the chondral structures, these, like the bone trabeculæ, undergo

absorption; and, depending upon the virulence and extensiveness of the infection, the joint cartilage undergoes local or complete absorption (Fig. 4). With the absorption of the cartilage the intra-articular structures become involved in the morbid process.

The location of the central epiphyseal focus, and its tendency to extend, are subject to considerable variation. In the mild forms the process remains localized. Not infrequently the inflammatory area originates near the joint and extends towards the cartilage, finally penetrating this structure, within a more or less circumscribed area, without involving the epiphysis of the cartilage extensively. There are, on the other hand, cases in which the epiphyseal structure is extensively involved, though the process does not extend to the joint cartilage. Thus it may happen that the epiphysis is extensively rarefied and subsequently (due to static condition) becomes much deformed, though the joint structures are not at all involved. Again, there may be a circumscribed inflamed area more or less centrally localized which, when the lymphoid tissue undergoes degeneration, subsequently becomes converted to a cavity. Thus are formed cyst-like structures with variable content which are strikingly similar to the epiphyseal bone cysts found in the human subject. Such areas may subsequently become replaced by connective tissue, or connective tissue may invade the greatly enlarged marrow spaces, and thus are formed the so-called fibroid nodules or fibroid marrow.

The intensity and the rapidity with which the process extends varies greatly. In some cases the condition runs its course within a very short time, while in others it is very chronic and only very gradually extends to the neighboring areas. Not only are the intensity and the extent of the inflammation variable in themselves, but the relation to each other also varies decidedly. There may be an intense inflammation which may remain circumscribed, either involving the joint cartilage not at all, or only within a circumscribed area, or an inflammation apparently not nearly as

intense may in some cases extend diffusely throughout the epiphysis.

Unless there is a very intense and diffuse involvement of the epiphysis the condition may undergo resolution at any stage. The congestion subsides, the marrow cells are gradually reduced to the usual numbers, and the lost trabeculæ, though the bone remains rarefied for a time, are finally regenerated and the epiphysis resumes its normal density. When, however, the process has reached certain proportions complete restoration to the normal seems impossible. The marrow cells in the greatly enlarged marrow spaces undergo degeneration and the latter become filled with fat or connective tissue, or a thin, mucilaginous material; and the normal epiphyseal structures are replaced by a few large, or a number of small, cyst-like cavities. Subsequently even the mucilaginous content may disappear, and the epiphysis is entirely obliterated.

Not infrequently the lymphoid marrow near the joint surface becomes converted into granulation tissue, which enters the joint interior through a gap in the joint cartilage. Should the condition continue to be active, the granulation tissue which has entered the joint interior gradually or rapidly spreads, filling more or less completely the joint cleft, the final outcome depending upon a number of contingencies.

The most important of these is the condition in the adjacent epiphysis and the joint capsule before the granulation tissue or the microörganisms have invaded the joint.

It is, of course, not to be denied that the microörganisms entering the joint interior with connective tissue, or with the new-formed vessels and lymphoid marrow which has perforated the cartilage, may directly invade the synovial membrane and thus produce inflammatory changes in this structure. I have not, however, been able to definitely establish this fact. In the cases I have examined streptococci could rarely be demonstrated in the synovial membrane even when there was involvement of the interior of the joint and reactive inflammation of this structure, unless there was a subperiosteal

focus from which the microörganism directly invaded the capsule.

In some instances the joint cleft was obliterated by granulation tissue or dense connective tissue, though the cartilage of the adjoining epiphysis was normal to all microscopic appearance. More frequently, however, the adjoining epiphysis also contained infective foci, or showed changes which indicated that such foci had existed. In some cases there was instead of a joint cleft a continuous layer of granulation or connective tissue between the epiphyseal marrow of the two articulating bones without a vestige of cartilage between them. Occasionally I was able to demonstrate ossifying areas in the connective tissue which bound the adjacent epiphyses together. And though I have not as yet been able to follow the process to its completion, it seems to me plausible that it is the gradual extension of ossification in the proliferating marrow which leads to bony ankylosis. From local and general synarthrosis to those cases in which there are signs of ultimate bony ankylosis, the process may become quiescent at any stage, and thus varying degrees of joint disability and joint ankylosis may be induced.

The subperiosteal epiphyseal focus begins as a localized inflammatory area in the region near the capsular insertion. In a very short time this localized inflammation has led to local periostitis. In the milder cases the disease subsides at this stage and the clinical phenomena of capsule irritation, joint effusion, and peri- and para-articular edema disappear completely, and there remains a localized periosteal thickening or node as the only permanent change.

When the disease advances from the periosteum to the capsule, an event which frequently occurs, even when the central epiphyseal focus remains localized, the inflammation gradually invades the joint capsule. Here hyperemia is succeeded by the usual phenomena of inflammation, round cell infiltration, finally granulation and connective tissue formation, leading to marked thickening of the capsule with or without proliferation of the fixed tissue cells. The capsule covered with granulation tissue may become adherent

to the joint surface and subsequently the cartilage becomes invaded by vascular loops and granulation tissue (the so-called pannus). As a general rule, however, the inflammation invades the joint surface from the transitional area between the capsule cartilage and periosteum. Thence vascular loops which are soon followed by inflammatory tissue gradually spread over the surface of the cartilage. When the inflammatory condition is mild the cartilage resists the invasion of this so-called pannus. When, however, the condition is intense, or continues for a considerable period, the cartilage finally becomes invaded and absorbed. Naturally the absorption of the cartilage varies with the extent and distribution of the morbid process. It may be circumscribed, producing a condition more or less aptly designated as an ulceration; it may be general and intense, the joint cleft completely obliterated and the opposing joint surface bound together by granulation tissue, or in the final stages by connective tissue (fibrous ankylosis). On the other hand, the inflammatory condition may remain entirely superficial, and the joint surface simply covered with more or less dense non-vascular connective tissue without destruction of the cartilage in the final stage (Figs. 5 and 6).

It is evident, therefore, that both as a result of subchondral and periosteal invasion the inflammatory conditions to be found in streptococcus infections in dogs, like the inflammatory conditions in man, are subject to great variations, these variations being due to variations in the intensity, localization, derivation of the disease, and the stage when the tissues are examined.

The condition is somewhat modified by the original location of the inflammation in the subperiosteal region on the one hand, and the subchondral region on the other. But this only applies to early or localized conditions. In the active forms the infective granulations coming from the marrow, even when they have invaded the interior of the joint from a localized area only, finally cover the joint surface, fill the joint cleft, and invade the cartilage of the opposing joint surface and the synovial lining of the capsule. Thus

either synovial forms or osseous forms of infection may maintain their original characteristics or they may, and often do, become converted into panarthritides.

Hence it is evident that inflammatory conditions which closely resemble those which occur in the human subject can be induced in dogs as the result of streptococcus infections. These conditions not only resemble those to be found in the known infections, but they correspond fundamentally to the changes found in the joints of those suffering from chronic joint disease of unknown origin, when these joints are examined during the active stage of the disease.

The pathogenesis of these conditions is readily followed in the dog with experimental streptococcus infection. The so-called synovial forms of the disease are evidently due to subperiosteal foci which have not become converted into panarthrites. The osseous forms are due to subchondral foci which have either caused only localized penetration, or have not penetrated the cartilage at all.

The fact that in cases of so-called arthritis deformans, marked deformation may occur with only slight or no change in the cartilage, is readily explained by the widespread softening and bone absorption which occurs during the active stages of the subchondral infection. It is not surprising that, under such circumstances, static and mechanical conditions should induce deformation of the epiphysis, which remains permanent though the active disease subsides. In one dog characteristic deformation of the head of the humerus was caused by subchondral infection without involvement of the joint capsule or the joint interior. That the structure of such an epiphysis may finally become readjusted to accommodate itself to the mechanical conditions to which it is subservient is a matter which should require no discussion.

But it is not only possible to follow the changes analogous to those inflammatory conditions which lead to joint abnormalities in the human subject; the peculiar changes in the tissue cells, which so long mystified pathologists and were ascribed to specific morbid influences, are also to be

found in joint structures of the dog inoculated with streptococci. The affected animals were not allowed to live long enough for the development of osteophytes and echondroses: the forerunners of such formations are, however, readily demonstrable. Thus proliferation of the cells in the transitional cartilage periosteum and capsular area to form connective tissue or cartilage cells, and the subperiosteal cell proliferation to form cartilage are not infrequent concomitants of streptococcus infection (Fig. 7). Moreover, the connective tissue proliferation, nodular and diffuse, within the marrow of the epiphysis — conditions which correspond to those found in the human subject in a number of different joint and bone diseases — are often found in the final stages of streptococcus infections in the dog (Fig. 8).

The part played by the joint cartilage in the inflammatory process must be briefly considered. In one of my earlier papers on the pathology of joint disease it was stated that the joint cartilage plays a passive part in joint disease, irrespective of the origin or the course. Later the experience in human pathology and the experiments just described have confirmed this view. The cartilage may be absorbed from above or below; it may be completely replaced by granulation tissue (Fig. 5); the absorption from above or below may be circumscribed, causing simply a defect; isolated areas of apparently normal cartilage may remain within granulating or proliferating marrow tissue, or more or less normal cartilage may exist under a layer of more or less vascular connective tissue (Fig. 6). But the cartilage itself never partakes of the inflammatory reaction. Like bone, it is passive and makes way for proliferating soft tissue.

The experiments referred to above were repeated with another strain of streptococci; with pneumococci and with staphylococci. Aside from minor differences in symptoms and course (staphylococci caused more acute conditions with and without suppuration, with a tendency to involve the shaft), the infections caused by these microörganisms were followed by analogous changes in the joints. Because they were so similar in results the experiments are not described

in detail. Twelve dogs were inoculated with pneumococci and twelve with staphylococci according to the usual laboratory technic. With the staphylococcus, two cases developed osteomyelitis with findings in no way different from those described by Lexor.

Thus the experimental evidence bears out the conclusions drawn from the pathology of human joint disease. It must, therefore, be concluded that in spite of the apparent variations the principal gross and microscopical findings in both acute and chronic joint disease, whether of known or unknown origin, are fundamentally simply phenomena of an inflammatory reaction. And as all pathological phenomena known to be characteristic of so-called arthritis deformans and rheumatoid arthritis are analogous to conditions due to known infection and can be reproduced experimentally by inoculating animals with pathogenic microörganisms, it must either be assumed that practically all forms of morbid influence induce fundamentally the same changes in the joint structures, or that these conditions are due to infections.

However, though they are evidently not characteristic of definite morbid entities, and the final outcome is in many cases a panarthritis, the lesions in the joints may for the sake of convenience be divided into degenerative or atrophic and proliferative types, when the conditions characteristic of such type predominate in a particular joint. Moreover, as will be shown later, for clinical as well as anatomical purposes it is important to differentiate the lesions in the joints according to the location of the disease. Thus osseous forms of joint disease are to be distinguished from synovial forms, though as has been shown the synovial forms are, as a matter of fact, in all probability always due to subperiosteal foci—*i.e.*, are really of osseous origin. However, as subperiosteal and synovial disease may exist without actual bone involvement, not only in the conditions due to the so-called septic bacteria, but also in cases due to the tuberculous bacteria and syphilis, and as these conditions induce a more or less definite pathological and clinical symptom complex, it is advisable to distinguish this form of joint involvement from the definitely

osseous forms. The more so, as has been demonstrated in my experiments and verified by clinical and pathological experience in the human subject, an epiphyseal focus may remain within the epiphysis without invading the joint interior or injuring the joint cartilage, thus inducing characteristic pathological and clinical findings. Such lesions are not infrequently found both in acute as well as chronic joint disease, and they are not at all uncommon in chronic monarticular disease due to the tubercle bacillus and other infective suppurative and non-suppurative conditions.

Hence, though I have been forced to the conclusion that these conditions are not due to definite morbid entities or specific forms of infections, I still find the classifications of the joint diseases I proposed some years ago, when somewhat modified, useful for both clinical and pathological demonstrations.

All forms of arthritis are due to inflammations of infectious origin. These may be divided according to the course of the disease into acute and chronic: according to the pathological lesions into active inflammations and proliferations and degenerations; and according to the location of the disease into osseous and subperiosteal or synovial forms of disease.

In some cases the disease runs true to type throughout its course; in others the final outcome is a more or less complete panarthritis. None the less when it is remembered that the division is more or less arbitrary; that the actual lesions are simply variations of a pathological process due to a great number of different etiological factors; and that transitional forms are common, this classification is of decided value as an aid to the study of both acute and chronic joint diseases.

Conceding for the moment that the pathological phenomena of all forms of joint disease can be reproduced in animals by experimental infection, and that it is not the specificity of the microörganism, but its virulence, the resistance of the host and its location, which determine the variations of the morbid process, it still remains to be shown

that all the clinical conditions met with in the human subject correspond to this conception of the pathology of polyarthritis.

There is no doubt the general symptoms in a large number of cases correspond with those of the infected animals. Thus the acute evanescent sterile intra- and peri-articular joint effusion characteristic of so-called acute and articular rheumatism, and the acute and sub-acute arthritides frequently present as complications or sequelæ of typhoid fever, gonorrhea, pneumonia, scarlet fever, etc., correspond exactly with acute experimental arthritis in the dog. The foci in the joints may or may not be accompanied by foci elsewhere (endocardium, myocardium or kidney), and for this reason we have polyarthritis with or without endocarditis, myocarditis, or nephritis, etc. We have, moreover, in the human subject, just as in the dog, cases with acute or sub-acute onset with subsidence of the constitutional disturbance, but progressive mono- or poly-articular joint disease. That is, the morbid process becomes localized in the joint structures. In such cases the onset may, for reasons stated in a previous paper, be entirely overlooked, and the course so insidiously progressive that the infectious nature of the disease is unrecognized.

In the human subject we find, besides recurrent infections which may be followed by complete restoration, progressive joint disease, or a variable amount of permanent, but not progressive alteration of the structures involved. In such cases the assumption that a focus either in the joint or elsewhere, after becoming quiescent, again resumes its activity, producing constitutional symptoms and metastases, seems perfectly justifiable.

Such conditions could not be definitely reproduced in animals. But in the dog the disease caused by streptococcus, pneumococcus, and staphylococcus inoculations presents the same capriciousness as regards the onset, the number of joints involved, and the subsequent course, which are characteristic of the chronic non-suppurative polyarthritis in the human subject.

In man the local clinical phenomena of the articular disease correspond in a great many respects to those of experimental arthritides in animals. The decalcification and rarefication caused by the vascularization and marrow proliferation can be demonstrated by radiographs and even the distinct subperiosteal and subchondral foci are often demonstrable in the radiographs of the fingers in the early stages of the sub-acute articular infections of the human subject (Figs. 9 and 10). In some cases, moreover, such subchondral and subperiosteal foci remain localized. Thus the so-called Herberden's nodes are no doubt analogous to the permanent para-articular periosteal thickenings found in the dog, and more or less permanently localized subchondral disease, though more common in chronic monarticular disease, are not at all rarely met with in both acute and chronic polyarthritis. We see them in the radiographs of many patients in the early stages of the monarticular forms of chronic arthritis and I have not infrequently been able to demonstrate exactly the same circumscribed foci of inflammation in some of the joints in cases of chronic, nonsuppurative polyarthritis. Figure 11 is the radiograph of the hip with such focus in a case of polyarticular gonococcus infection and Figure 12 depicts one from a case of general infection following a prophylactic typhoid inoculation, and Figure 13 a circumscribed focus in the neck of the femur, due either to tuberculosis or another unknown form of infection.

Clinically the subperiosteal and subchondral foci may run true to type throughout the course of the disease. There are cases of joint disease in which the subperiosteal focus remains within the soft structures and never involves the bone. Such conditions are not very uncommon, particularly in tuberculosis of the knee, and analogous conditions with a definite clinical picture are also induced by other micro-organisms. That these are not really due to primary synovial involvement does not make them the less characteristic.

Definite subchondral foci which never penetrate the cartilage and never cause marked intra-articular proliferative phenomena occur and induce characteristic symptoms (joint

spasm in the early stages, and loss of motion which corresponds to the deformation of the articular ends of the bones, due to static influences during the stage of decalcification and rarefication) and though not common are easily demonstrable in all forms of infection.

Whether the softening and absorption is accompanied by the so-called proliferative processes depends upon the virulence of the microörganism, the resistance of the host, and the mechanical conditions which prevail in each particular case. If the condition remains subchondral, the epiphyseal trabeculæ may be restored; when depending upon static conditions during the active period of the disease, there remains permanent deformation or restoration of the normal anatomical contours.

In those joints in which the inflammation is followed by degeneration, or because of the loss of surface conformation, muscle shortening, muscular atrophy, or capsular contraction, motion is impossible, greatly impeded, or its resumption, after the active process has subsided, unduly delayed, the atrophy which appears during the early stages becomes more or less permanent. The atrophy under these circumstances is due not only to the original inflammation, but to disuse as well. Such conditions may be entirely unaccompanied by proliferative phenomena, and it is under these circumstances that the conditions known as the atrophic forms of joint disease are induced, all of which I formerly believed to be due to metabolic disturbances.

In correspondence with the pathological findings in experimental and human joint disease the clinical conditions, in the majority of cases, which do not undergo resolution during the early stages, assume the characteristics of a panarthritis. When the active process has subsided, the local symptoms depend upon the extent and location of the joint destruction and the presence of fibrous or bony adhesions.

However, though the clinical phenomena of the actual joint disease correspond exactly to the morbid changes reproduced in the joints of animals experimentally inoculated with various pathogenic microörganisms, there still remain

certain clinical phenomena frequently present in human polyarthritis which cannot be definitely associated with the conditions as they occur in the experimental arthritis.

It has not been possible, for instance, to reproduce in animals those forms of polyarthritis which apparently invade the peripheral joints first and then gradually extend to the proximal articulations. But polyarthritis of this type is due to a number of factors not correlated to the etiology of the disease. In man the movements of the hands and fingers are not only finely adjusted and complicated, but they are almost constantly employed. Hence derangement of function and pathological conditions generally, which in other situations are hardly appreciated, produce conspicuous symptoms in the hand and fingers. Obviously such conditions cannot be reproduced in animals in whom the hand is entirely lacking. Moreover, as my facilities for carefully observing a large number of cases of so-called arthritis deformans or rheumatoid arthritis have increased, I have become more and more convinced that, though the symptoms in the hands are most conspicuous, the disease has, in many of the cases which have apparently begun peripherally, involved other joints simultaneously. There are, no doubt, cases in which the disease attacks the peripheral articulations first and then apparently spreads to the larger joints, but as both the intrinsic pathological and clinical phenomena in these cases are analogous to those found in other joints, in cases which do not present symmetry of invasion or a primary peripheral involvement it must be concluded that these peculiarities are due to accidents of localization.

There are on the other hand certain peculiarities characteristic of so-called arthritis deformans which are apparently very difficult to bring in correlation with an infectious origin. Indeed the peculiar deformations of the hands and fingers, the akroparesthesia and the premonitory weakness and spasm cannot be ascribed to the infectious focus within the joint at all. Many theories as to the origin and the cause of these peculiarities have been advanced, but none of them have satisfactorily explained the presences and mechanism of

these conditions. Careful study of the peculiar deformation, so graphically described by Charcot and said by him to be due to reflex irritation of the anterior horn cells, reveals the fact that these conditions cannot be ascribed to a definite single cause or mechanism.

As has been shown in a previous paper, the akroparesthesia, the spastic weakness, and the contractures which are present in many of the more severe cases of so-called arthritis deformans are in all probability due to a coincident spondylitis and secondary involvement of the cord or the spinal roots. Besides the symptoms and objective changes indicative of central involvement, there are in many cases definite signs of peripheral nerve abnormalities. In many of these cases the symptoms simply indicate nerve irritation or at most a perineuritis. But in some there are the classical signs of more or less localized peripheral neuritis. Thus Figure 14 is the hand in the case of polyarthritis showing the characteristic contracture and trophic disturbance of ulnar neuritis. In this case though many other joints were involved and there were originally some symptoms of arthritis in this hand, there now only remains the characteristic changes of the neural involvement. In Figure 15 the more or less characteristic main en griffe of ulnar neuritis with the drop wrists of radial neuritis is perfectly evident. Figure 16 illustrates the peculiar deformity which follows median neuritis. In this case as the radiograph shows (Fig. 17), though there is dislocation of the first phalanges upon the metacarpals and there are definite signs of arthritis in the peripheral phalangeal joints and the carpus, there are no bony changes in the metacarpo-phalangeal joints.

In some of these cases arthritic changes are present in the joints which have evidently been deformed by the contractures due to the neuritis and thus the deformity may (as in the wrist of Fig. 15) become fixed by fibrous or bony ankylosis.

The fact that there may be peripheral and central neural involvement in polyarthritis not only accounts for many of the peculiar forms of deformation, particularly of the hands

and the fingers, but it readily explains the changes in the skin — glossy skin ulceration of the skin and nails, the edema and the anomalies of sweat secretion so common in these cases. These neurotic changes and the deformities and skin changes caused by them affect not only the hands and fingers, but may involve other peripheral and proximal parts of the extremities. Thus the deformities which occur in polyarthritis are caused not only by joint changes which result in more or less complete loss of motion, but by neuro-muscular changes as well. Either may exist to the exclusion of the other or combinations of the two may co-exist in the same joint or in a number of joints in cases of polyarthritis.

That the neural changes may be and in all probability are due to central or peripheral neurotic changes is borne out not only by the fact that such peripheral neuritides are not uncommon in infections without joint involvement, but by the results of our experimental inoculations in animals in which I have been able to demonstrate the involvement both of the central and peripheral nerves.

The deformities which occur as the result of various combinations of arthritic and neuro-muscular involvement are numerous and often grotesque, and upon this basis many of the more remarkable features of the so-called arthritis may be explained. There are, however, still other forms of deformity which are due to other causes, for not all the cases of subluxation and dislocation are due to contracture following neuritis or are not due to this cause alone.

We have (as in the photograph of the hand reproduced as Figs. 18 and 19) cases in which the later stages of the joint disease show complete dislocation of the phalanges, though the power is not affected the deformity does not correspond to peripheral or central nerve involvement, and the radiographs (Fig. 20 radiograph of the hand shown in Figs. 18 and 19) show no bone changes whatsoever. When one is familiar with the condition during the early stages of such a case the cause of this peculiarity is not difficult to explain.

As has been shown, the early stages of joint infection are characterized by intra- and peri-articular effusion. In some

cases the effusion is enormous. Thus, in the hand shown in Figure 21 the effusion has led to marked swelling not only of the phalangeal and metacarpo-phalangeal joints, but of all the tissues between these joints as well. Figure 22 shows the knees of a case in which the effusion, great as it is, has already been reduced by tapping.

An effusion which reaches great proportions always leads to relaxation of the capsule and peri-articular structures and (at times painless) dislocation of the joints. Such dislocations are common in typhoid fever. I have seen them in influenza and streptococcic infections. Thus Figure 23 is the radiograph showing dislocation of both hips as a result of an effusion in a case of general infection due to streptococcus.

The subluxation and dislocations caused by an exudate may occur without either bone or nerve involvement. They may, however, be combined with either or both. Thus Figure 24 is an illustration of the hand and Figure 25 the radiograph from a case with dislocation as the result of an exudate, combined with the deformity of a median neuritis. And dislocation combined with more or less complete destruction of the articular surfaces of the bone is illustrated in the metacarpo phalangeal joints in Figure 26. In such cases the exudate produces relaxation of all the tissues of the joints and prevents ankylosis. Hence there is often considerable passive or even voluntary motion in the affected joints in spite of the extensive destruction and dislocation.

It is to be noted that the actual neuritis may subside, but the deformity (as in other forms of muscular spasm) often remains permanent because of permanent muscle shortening. In such cases a fair amount of even voluntary motion in the dislocated or partly destroyed articulations is possible.

As the joint lesions and the nerve lesions in those cases correspond exactly to the lesions in evidently infectious conditions; as such lesions have been reproduced experimentally; and as these peculiar conditions have been shown to be due to the objective changes resulting from such lesions, I think the assumption justified, that these conditions are a

part of the symptom-complex of chronic non-suppurative infectious polyarthritis.

It is then fairly certain that all the various forms of polyarthritis may be caused by infections. The pathological lesions correspond exactly with those of known infections and the clinical phenomena can all be ascribed to differences in the location and virulence of the bacteria, the mechanical conditions and the presence or absence of central or peripheral nerve involvement. The classification of the so-called arthritis deformans into definite infections and problematical metabolic disturbances is therefore no longer necessary as far as the joint conditions are concerned. It must either be assumed that all forms of polyarthritis are due to infections or that all deleterious substances, whatever their nature, will cause fundamentally the same general changes in the articular structures. And it cannot be denied that there is some foundation for this interpretation of these phenomena. For it is well known that traumatic conditions near or in the epiphysis induce the phenomena of inflammation, congestion, vascularization, bone decalcification and absorption, and at times tissue proliferation which may lead to fibrous or bone ankylosis. However, from our clinical observations and despite this analogy, which is true of inflammatory conditions generally, I think that we must, for the present at any rate, conclude that in at least the majority of cases the cause of the chronic, non-suppurative joint diseases is an infection. The question as to the exact nature of the invading microorganism must still remain doubtful in many cases. Though the various forms of streptococci are the offending species in probably a large number of cases, it must be admitted that similar or analogous joint changes may be experimentally induced by other microörganisms and that not infrequently gonococci or staphylococci have been known to induce such conditions in the human subject.

For clinical, and especially therapeutic purposes I believe we must consider the disease from its general and local aspect. Thus the presence or absence of symptoms of general

infection, the metastases in other organs besides the joints, the recurrences and the location of the primary focus must be considered as a problem separate from the articular disease.

In this regard a word of caution is indicated. Though it is not unlikely that a focus in a tooth is sometimes and one in the throat is often the point of entry for bacteria, it should be remembered that once the microörganism has entered the blood its connection with the portal of entry ceases. For this reason, though the removal of the affected teeth or tonsils will, if these are really the site of the original focus (which is not by any means always certain even when they are abnormal) prevent reinfection or recurrences, such procedures have absolutely no influence upon the joint condition as it already exists. I am afraid that this fact is seldom made clear to patients who are persuaded to resort to surgical measures in these organs. Moreover, it must be remembered that a focus in the joint structures, like a focus anywhere else, besides causing local changes, may be and no doubt often is a source of general infection and metastases.

The local articular conditions must be divided into active and terminal stages. The active stage may be short and terminate in complete resolution or it may last a very long time; that is it may be chronic. In the chronic conditions it is often of importance, in view of treatment, to differentiate between epiphyseal and subperiosteal or synovial forms of disease. In the final stage the terminal condition to be met with is more or less readily differentiated by the objective signs and the radiograph. Thus we can often distinguish between degenerations without loss of passive motion and more or less deformity (dislocation, etc.), fibrous ankylosis, bony ankylosis. It should be remembered that these changes are not typical of a certain class of cases, but may and do occur simultaneously in different joints in the same individual. Finally the presence or absence of concomitant central or peripheral neural involvement must be determined.

When the fact that the joint condition — either non-articular or polyarticular — may remain active and progressive though the general condition has subsided, that the local joint condition varies according to the virulence (but not the cause of the infection), the termination, the mechanical conditions in the joint and the concomitant conditions, there need no longer be any difficulty in understanding and caring for the so-called rheumatoid polyarthritides.

In conclusion I can say only a few words as regards the treatment of the conditions here discussed. Some years ago I strongly advocated the use of thymus extract in the treatment of these diseases. At that time I stated that this substance is not a specific, and from what has been said in the foregoing pages there can of course be no doubt in regard to this. The fact nevertheless remains that thymus seems to have a very definite beneficial effect upon the nutrition and I still find that in those cases in which the joints are not destroyed or ankylosed (providing it is long continued and the routine dieting and mechanical treatment which are so harmful are omitted), it nearly always leads to more or less complete recovery.

When the mechanical functions of the joints are impaired the problem becomes a mechanical one, depending upon the mechanical conditions not only in each case, but in each joint.

[The work was done in the Carnegie Laboratory, and I cannot sufficiently express my obligation to Dr. Symmers, who placed the pathological laboratory and the necessary material at my disposal. I am indebted to Dr. Noble of the bacteriological department of the Carnegie Laboratory, and to Dr. Celler and Dr. Olitzky of the pathological laboratory of the Mount Sinai Hospital for cultures of microörganisms. I am also indebted to Dr. Schultz of the Montefiore Home and Hospital for the radiographs.]

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DESCRIPTION OF PLATES XII.–XVII.

Fig. 1. — Articular end of head of the femur from a case of multiple infectious arthritis, showing: A, subchondral cyst; B, mass of fat completely replacing the marrow and bone trabeculæ.

Fig. 2. — Microscopical section of articular end of the head of the femur shown in Figure 1: A, thin layer of non-vascular connective tissue covering joint cartilage; B, in which the cells are degenerated; C, granulation and fat tissue. The bone trabeculæ have almost completely disappeared.

Fig. 3. — Early stage of experimental arthritis. The marrow spaces are almost completely filled with lymphoid marrow containing numerous giant cells.

Fig. 4. — The articular end of the lower end of the femur from a case of experimental polyarthritis. The bone trabeculæ are much diminished and the lymphoid marrow has just begun to invade the joint cartilage. The cartilage cells are normal.

Fig. 5. — Experimental arthritis, final stage. The acetabulum of the hip joint. The cartilage has been completely absorbed and the articular surface consists of connective tissue and the remains of the so-called pannus.

FIG. 6. — Experimental arthritis, final stage. Upper end of tibia. The articular surface is covered by a layer of non-vascular connective tissue. The underlying cartilage cells are apparently normal.

FIG. 7. — Experimental arthritis. Tibia. Cartilage cell proliferation under the periosteum.

FIG. 8. — Endosteal marrow changes in experimental arthritis: A, cyst formation; B, fibrous degeneration of the marrow; C, cartilage cells in the marrow.

FIG. 9. — Subchondral foci in the phalanges of index finger in a case of sub-acute infectious arthritis.

FIG. 10. — Subperiosteal changes in the early stage of a case of chronic infectious arthritis.

FIG. 11. — Subchondral focus in the head of the femur in a case of chronic polyarticular gonococcus infection.

FIG. 12. — Subchondral focus in the head of the femur in a case of general infection following prophylactic typhoid inoculation.

FIG. 13. — Focus in the neck of the femur due to some unknown micro-organism, probably the tubercle bacillus.

FIG. 14. — The characteristic deformity and trophic skin disturbance of an ulnar neuritis in a case of chronic polyarthritis.

FIG. 15. — Main en griffe complicated with the drop wrist of radial neuritis in a case of chronic polyarthritis.

FIG. 16. — The characteristic deformity of median neuritis in a case of chronic polyarthritis.

FIG. 17. — The radiograph of the hand shown in Figure 16. The first phalanges though dislocated by contracture show no bone involvement. The more peripheral phalangeal joints and the carpus show bone and joint involvement with ankylosis.

FIGS. 18 and 19. — Show the deformity due to dislocation without bone involvement and without loss of voluntary power.

FIG. 20. — Radiograph of the hand shown in Figures 18 and 19. The first phalanges are completely dislocated, but there is no bone involvement.

FIG. 21. — Marked intra-, peri-, and para-articular exudate. Such conditions often lead to dislocation.

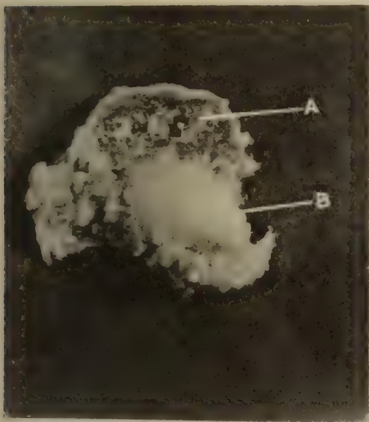
FIG. 22. — Marked intra- and peri-articular effusion in the knee causing relaxation of the peri-articular tissues.

FIG. 23. — Complete dislocation of both hips as the result of an exudate in a case of general infection due to streptococcus.

FIG. 24. — Hand showing the deformity caused by an inflammatory exudate combined with ulnar neuritis.

FIG. 25. — The radiograph of the hand shown in Figure 24, showing that the dislocation is not due to bone involvement.

FIG. 26. — Radiograph of the hand which shows the result produced by dislocation due to the exudate when combined with absorption of the articular ends of the bone.



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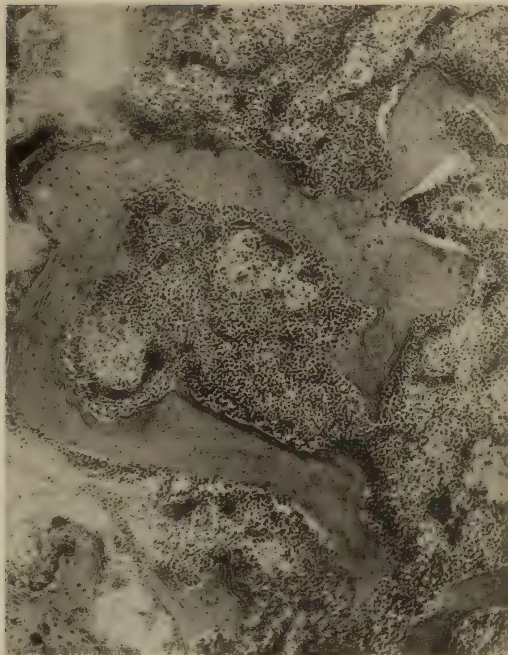

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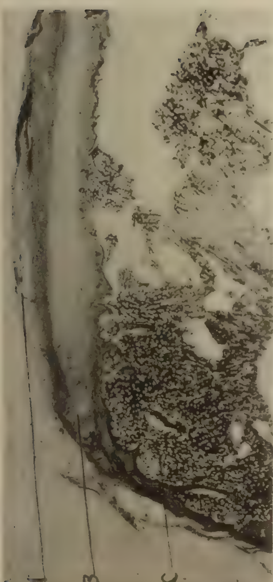

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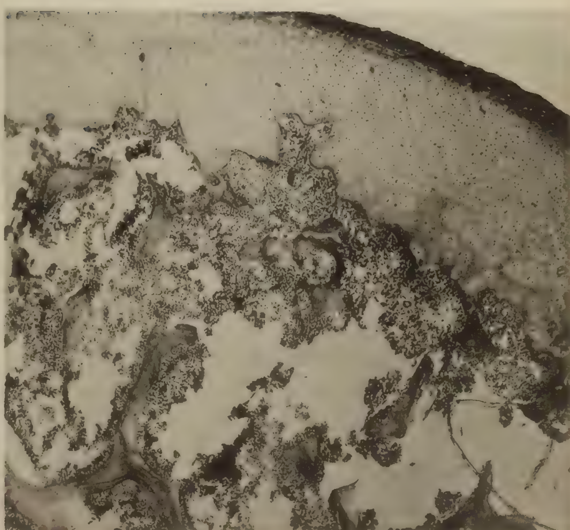

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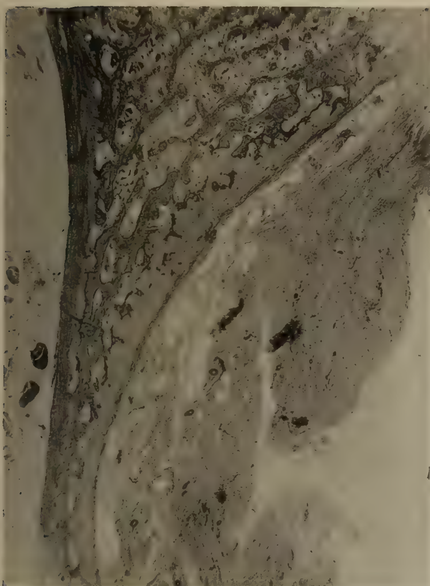

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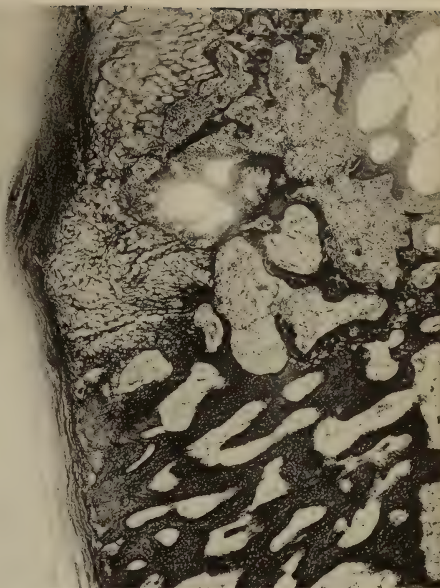

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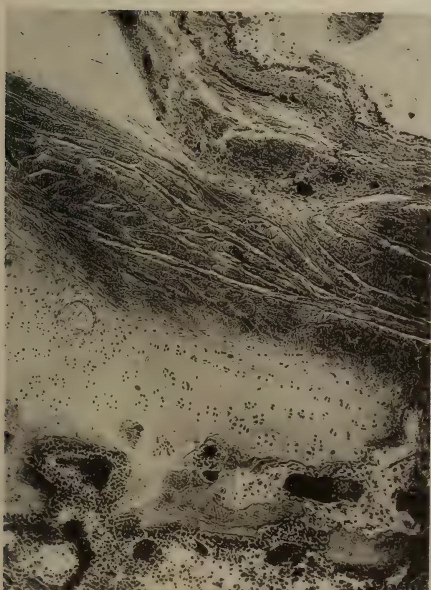

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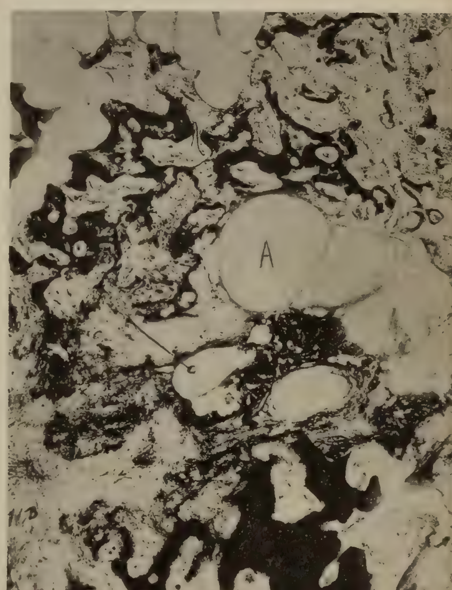

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OBSERVATIONS ON THE FORMATION OF GIANT CELLS IN TUBERCULOSIS.*

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The giant cell found in the characteristic pathological lesion of tuberculosis was first accurately described by Langhans [9] in 1868. Since that time many investigations have been made concerning the origin, function, and fate of these cells. It would be impracticable to review the details of this work on account of its volume. Many of the references cited below give reviews and extensive bibliographies.

As a result of the numerous investigations there have arisen several disputed points. It will only be necessary to enumerate the more important ones. First, the giant cells have their origin from fixed connective tissue cells or epithelial cells — Baumgarten,[1] Klebs,[6] Oppenheimer,[12] Miller,[11] Watanabe,[16] and others; or they are of leucocytic origin — Metchnikoff[13] and his followers. Second, the giant cells are formed by a fusion of cells — Kostenitsch and Wolkow,[8] Kockel,[7] Miller,[11] Watanabe,[16] Metchnikoff,[13] Walgren;[15] or they arise by repeated division of the nucleus of a single cell without a division in its cytoplasm — Weigert,[17] Straus,[14] Oppenheimer.[12] Third, the giant cells are destroyed — Baumgarten,[1] Weigert;[17] or they may subdivide into many cells and take on various forms such as fixed connective tissue cells — Hektoen,[5] lining of blood vessels — Klebs,[6] or even the lining of the alveoli of the lungs — Klebs.[6] Fourth, the giant cells are actively phagocytic — Metchnikoff and many others; or they are simply an accompanying feature of necrosis — Baumgarten,[1] Weigert.[17]

Mallory[10] believes the giant cells in tuberculosis to be formed by a fusion of endothelial leucocytes. Very recently Evans, Bowman, and Winternitz[3] have produced tubercles in vitally-stained animals and thus demonstrated that the cells

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making up the tubercle and the giant cells were derived from the endothelial lining (Kupffer's cells) of the sinusoids of the liver.

Many methods of staining have been employed in attacking this problem, but so far as I am aware the silver impregnation method of Bielschowsky has never been used. The specificity of this method for reticular fibrils has apparently been well established. Ferguson,[4] after a careful investigation of this method, came to the conclusion that excluding muscles, nerves, and embryonic mesenschyme it was specific for the reticulum of Mall. Downey[2] employed it in determining the origin and nature of the so-called endothelioid cell. He believes that this cell comes from the reticular cells lining the sinuses of the lymph nodes. Hence the term "reticulo-endothelial" leucocyte has come into vogue.

I have repeated this work of Downey's, using normal lymph nodes of kittens and have confirmed his findings. While doing this the question arose whether this method would throw some light on the question of the origin of giant cells and their mode of formation. This paper embodies the results as applied to the giant cells in tuberculosis. The material used in this study consists of cervical lymph glands and Fallopian tubes obtained at operation, and of various organs obtained at autopsies.

These specimens had been fixed in ten per cent formalin, alcohol, or Zenker's solution. Portions were embedded in paraffine and sections varying from four to eight microns were cut. In some cases serial sections were made. Besides using the Bielschowsky silver impregnation method as employed by Ferguson,[4] sections were stained with hematoxylin and eosin, iron alum hematoxylin, and Mallory's connective tissue stain, Mallory's phosphotungstic acid hematoxylin, and carbol-fuchsin.

Upon examination of a section after a successful silver impregnation, one can easily distinguish the black reticular fibrils from the brown-colored collagenous fibers (Fig. 1). The tubercles can be recognized easily, and the size, shape,

and nuclei of the cells can be discerned. With higher magnification and careful focusing one can see distinct fibrils in many cells that appear identical morphologically with the so-called epithelioid or endothelioid cells (Fig. 2). Some of these fibrils seem to extend beyond the cytoplasm and appear naked while others have a faint rim of cytoplasm surrounding them. The arrangement of these fibrils corresponds in extent to the distribution of the cells making up the tubercle (Fig. 1). This arrangement in the tubercle indicates that the fibrils are newly formed and do not represent the remains of the old reticulum. Often in the same section one can find tubercles of various ages, some just forming, others in the process of degeneration and caseation, while still others seem to be in the process of healing. In the latter, there is a distinct band of collagenous fibers around the periphery.

Giant cells are numerous in many of the sections. All stages of formation are to be found. In many of the smaller giant cells containing only a few irregularly-placed nuclei, there can be seen reticular fibrils arranged in such a manner as to indicate that they had been formed as a result of fusion of two, three, or more, of the above-described reticular cells (Fig. 3). The nuclei of these smaller giant cells present the same characteristics as those of the reticular cells. In many cases there is an indication of the original cell boundaries at the edge of the giant cell.

Two types of larger giant cells are found. First, those whose outlines are regular and whose nuclei are arranged around the periphery (typical Langhans giant cell). In this type there is no indication of reticular fibrils in the cytoplasm nor is there any indication of growth either by nuclear division or by the addition of other cells. In the second type, the outline is very irregular and the nuclei are scattered irregularly through the cytoplasm. Many of the prolongations of this type of cell are continuous with the cytoplasm of the surrounding cells, and often in these prolongations reticular fibrils can be seen. They present the general appearance of increasing in size by the addition of new cells.

A great many sections stained with iron alum hematoxylin were studied, but in none could a mitotic figure be seen in the giant cells.

From the above findings, one seems justified in believing that the so-called epithelioid and endothelioid cells are really of reticular tissue origin and also that the giant cells in tuberculosis are a result of the fusion of these cells.

These views do not differ greatly from the ones held by the investigators who believe that giant cells originate from the fixed connective tissue cells, neither do they differ greatly from the conclusions of Evans, Bowman, and Winternitz that the cells of the tubercle and the giant cells in the liver arise from the endothelial cells lining the sinusoids of the liver. Reticular fibrils have been demonstrated in the normal liver between the endothelial cells and the liver cells forming the tubules. As only endothelium, modified endothelium (Kupffer's cells), and liver cells are found in the liver lobule, it may be that one or both of the first two are concerned in elaborating these fibrils.

Whether the cells elaborating reticular fibrils that make up the tubercle and form the giant cells in the various organs of the body were carried in by the blood or lymph vessels was not determined. If this were the case one would expect to find an increase in the number of reticulo-endothelial leucocytes in the blood stream.

Since reticular tissue is found throughout the body, it would seem entirely possible that the cellular reaction in tuberculosis is entirely local, and due to a stimulation of these reticular cells.

SUMMARY.

The specificity of the silver impregnation method for reticular fibrils seems definitely established (Ferguson).

The demonstration of reticular fibrils in the cytoplasm of the so-called epithelioid or endothelioid cells in the tubercle, therefore, identifies them as of reticular tissue origin.

The presence of fibrils in the cytoplasm, the similar morphological characteristics of the nuclei, the absence of

nuclear divisions, and in some cases the existence of partial cell walls occurring in the smaller giant cells would seem to indicate that they are the result of a fusion of cells of reticular tissue origin.

[The author is indebted to Drs. Ernest Scott and Jonathan Forman of the Department of Pathology of Ohio State University for the material forming the basis of this study.]

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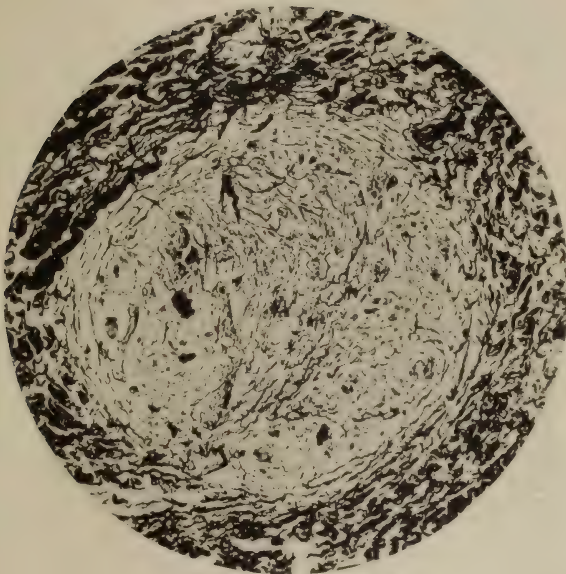
DESCRIPTION OF PLATE XVIII.

(All figures are photomicrographs of sections impregnated with silver following the method of Bielschowsky as employed by Ferguson.)

FIG. 1. — A small tubercle showing a typical arrangement of the reticular fibrils. The coarse fibers around the periphery are for the most part collagenous. x 135.

FIG. 2. — An early collection of cells that have fibrils in the cytoplasm. Some of the fibrils are deeply imbedded and closely associated with the nuclei while others are surrounded by only a faint rim of cytoplasm. x 1025.

FIG. 3. — Two small giant cells with irregularly-placed nuclei and reticular fibrils in the cytoplasm. In the right hand side of the lower giant cell the fibrils are in close association with one of the nuclei. x 675.



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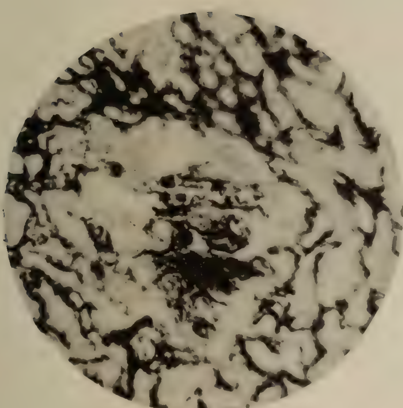

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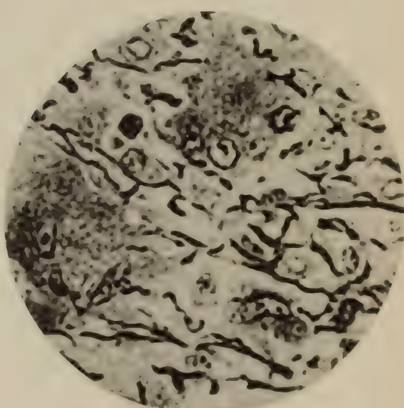

3

Warren. Giant Cells.

STUDY X.

Studies on the sensitization of patients with bronchial asthma to the proteins in animal, fruit, and vegetable foods.*

I. Chandler Walker, M.D., Boston, Mass.

(*From the Medical Clinic of the Peter Bent Brigham Hospital, Boston.*)

Three previous papers discuss the sensitization of patients with bronchial asthma to bacterial proteins (Study III.), to the proteins in the hair of the cat and the dog and in the dandruff of the horse (Study IV.), and to the proteins in wheat and to the whole proteins in the cereals (Study V.). The present paper is concerned with the sensitization of patients with bronchial asthma to the proteins in the common foods, exclusive of the cereals. The proteins which were discussed in the former papers and those discussed in this paper were tested on the same series of patients so that in a general way a comparison may be made between the frequent sensitization of patients with the proteins discussed in the three former papers and the infrequent sensitization of patients with the proteins in the common foods, exclusive of the cereals.

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* This is the tenth of a series of papers on the study of bronchial asthma made possible through a gift by Mr. Charles F. Choate, Jr., of Boston, to the Peter Bent Brigham Hospital. The papers previously published are: Study I., Studies with the Staphylococcus pyogenes aureus, albus, and citreus, and with the Micrococcus tetragenus and catarrhalis; Study II., Studies with a diphtheroid organism isolated from the sputum of patients with bronchial asthma; Study III., Studies on the sensitization of patients with bronchial asthma to bacterial proteins as demonstrated by the skin test and the methods employed in the preparation of these proteins; Study IV., Studies on the sensitization of patients with bronchial asthma to the different proteins found in the hair of the cat and dog and in the dandruff of the horse and to the sera of these animals; Study V., Studies on the sensitization of patients with bronchial asthma to the different proteins in wheat and to the whole protein of wheat, corn, rice, barley, rye, and oat. All in the Jour. Med. Research, 1917, Vol. XXXV., No. 3, pp. 373 to 402 and 487 to 513.

Study VI., Immunochemistry of the proteins of cat hair; Study VII., Immunochemistry of the proteins of horse dandruff; Study VIII., Immunochemistry of the proteins of dog hair. All in the Jour. Immunology, 1917, Vol. II., No. 3. Received for publication Feb. 15, 1917.

The method of preparation of the proteins from the vegetables and from the fruits has been described by Wodehouse.[1] The preparation of the serum proteins was described in Study IV. The proteins from fish and meat were prepared by the evaporation to dryness of a water extract of finely-ground fish and meat. The casein and the egg albumen used in the tests were obtained from a commercial house. The tests with wool, feathers, and hair were made with a twelve per cent alcoholic extract of these, as was done in Study IV.

The skin test, as was described in Studies III. and IV., was employed to demonstrate the sensitization to the proteins. Reactions consisting of an urticarial wheal and reactions consisting of a large area of erythema were considered to be positive; these types of reactions from the common food proteins closely simulated those produced by the bacterial proteins as described in Study III., but they were not usually as pronounced as were the reactions produced by the proteins from the cereals. Those reactions which are described as ± consist either of a very tiny elevation about the cut or of a small area of erythema; these reactions are considered as negative for diagnostic purposes, but they should be noted and repeated at another time since a second test may be positive or entirely negative. Those reactions which do not differ from the control-cut on which no protein is placed are called negative.

The first part of the following description shows the number of patients tested with the proteins of beef, chicken, lamp, egg, casein, and, for comparison, with the hair and the serum of cattle, with the feathers of chicken, and with the serum of sheep. The number of positive, doubtful, and negative reactions with each protein are given. In the second part the results on a few patients are given to show how patients may vary in their sensitization to the proteins found in different tissues or fluids of the same animal.

TABLE I.

The sensitization of patients with bronchial asthma to the hair, serum, meat, and casein of cattle, to the feathers, meat, and egg of chicken, and to the wool, serum, and meat of sheep.

	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Cattle hair	70	10	3	57
serum	35	2	2	31
beef	50	2	3	45
casein	60	1	3	56
Chicken feathers	70	4	4	62
meat	25	2	4	19
egg	60	1	3	56
Sheep wool	50	1	0	49
serum	20	0	0	20
lamb	50	0	3	47
Pork	30	0	0	30

In Table I. it is noted that fourteen per cent of those patients who were tested with cattle hair were positive, six per cent of those tested with feathers were positive, and two per cent of those tested with wool were positive. The importance of testing with these articles is realized when one considers the part they may play in house furnishings; cattle hair may find its way into upholstering and into mattresses, feathers may be present in pillows, and in women's neck ornaments, and wool is a part of blankets and clothing. Six per cent of those tested with cattle serum were positive, but no patient was positive with sheep serum. These sera may be ingested with meat which is cooked very rare. Of the patients who were tested with beef, four per cent were positive, of those tested with chicken meat, eight per cent were positive, and none of those tested with lamb and pork were positive. It should not be forgotten that these tests were made with the uncooked meats and that these same patients probably could eat thoroughly cooked meat without trouble. Only

one case in sixty reacted positively to egg and to casein; this is of interest when one compares Talbot's[2] findings, namely, that twenty-seven out of forty-five infants reacted positively with egg, and one out of forty-five reacted positively with casein.

TABLE II.

Patient.	Cattle.				Patient.	Chicken.		
	Hair.	Serum.	Meat.	Casein.		Feathers.	Meat.	Egg.
C. N. E. . .	+	O	O	O	H. T. . . .	O	+	O
J. H. N. . .	+	+	+	O	R. G. V. . .	+	O	O
H. T.	+	O	+	O	J. M.	+	O	+
W. M. O'D. .	O	O	O	+	A. C. . . .	±	+	O
R. A.	O	+	O	+				

Table II. illustrates how a patient may give a positive skin reaction to the protein from one tissue or fluid, and not from all of the different tissues or fluids of the same animal. Therefore, all proteins with which a patient may come in contact as well as those which a patient may eat or inhale should be tested before we can exclude protein sensitization in the individual.

TABLE III.

	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Halibut	25	O	3	22
Mackerel	25	O	2	23
Salmon	30	3	8	19
Lobster	30	2	8	20
Haddock	25	O	2	23

Table III. serves to compare the relative frequency between the number of positive reactions with salmon and lobster on the one hand, and the relative infrequency with haddock, mackerel, and halibut on the other side.

Table IV. shows the reactions obtained with the common vegetables. Potato is used practically as a routine test and pea and bean are tested frequently, but the remaining vegetables are used in tests only when a history of indigestion caused by them is obtained.

TABLE IV.

The sensitization of patients with bronchial asthma to the common vegetable proteins.

Vegetables.	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Potato	60	1	6	53
Pea	35	2	1	32
Bean	45	2	3	40
Carrots	8	0	0	8
Parsnips	13	0	0	13
Turnips	3	0	0	3
Squash	8	0	0	8
Celery	7	1	0	6
Asparagus	6	1	0	5
Beet	8	0	0	8
Spinach	17	5	6	6
Cucumber	4	0	0	4
Tomato	2	0	0	2

In the above table it is seen that potato, pea, bean, celery, and asparagus occasionally give positive skin reactions. The frequency of positive and doubtful reactions with spinach is probably due to some irritant in the preparation and we do not attribute the reactions to the protein, neither do we consider these reactions with spinach as diagnostic. The patients who reacted negatively to the remaining vegetable proteins gave a history of indigestion when they were eaten.

TABLE V.

The sensitization of patients with bronchial asthma to the proteins from the common fruits and nuts.

	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Strawberry	4	0	0	4
Orange	15	0	0	15
Banana	15	0	0	15
Apple	10	0	0	10
Pear	10	0	0	10
Walnut	10	0	1	9
Almond	10	0	1	9
Brazil nut	5	0	0	5

It is noted that no positive reactions were obtained with the proteins from fruits and nuts. The majority of the patients who were tested with these, however, gave a history of indigestion following the ingestion of them.

Conclusions. — In this series of patients with bronchial asthma only an occasional case was found to be sensitized to the proteins derived from the staple foods of animal, fruit, and vegetable kingdoms.

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STUDY XI.

Studies on the sensitization of patients with bronchial asthma to the various pollens.*

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For a long time it has been known that patients with bronchial asthma may be found to be sensitized to the various pollens and it is generally stated that sensitization to one pollen of a biological family means sensitization to all of the pollens of that family. The test for pollen sensitization is usually made only with dilutions of them. In our study on bronchial asthma we have found that sensitization to pollens does not always hold for the whole biological family, but that it is necessary to test with all the pollens belonging to that family. Furthermore, it is essential to use the whole pollen in the skin tests and if a positive reaction results the various dilutions of the pollen protein should be tested; if the dilutions alone are used many sensitized cases would be missed. Occasionally a patient who fails to give a skin reaction with the pollen of the plant will give a positive test with the leaves of that plant. The possibilities of sensitization to plants and to trees seem to be great.

While working on the methods of obtaining pollen from rag weed Wodehouse found that carbontetrachloride greatly facilitated the procedure and we have since found that this same method is applicable to all animophylous pollens. The flowers are collected just before they open and are then dried. The dry flowers are macerated with carbontetrachloride, pressed through gauze which allows the pollen and tetrachloride to escape; then the pollen is collected on filter paper. In a few hours the pollen on the filter paper becomes dry and can be removed as a fine powder; this is used for the

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* This is the eleventh of a series of papers on the study of bronchial asthma made possible through a gift by Mr. Charles F. Choate, Jr., of Boston to the Peter Bent Brigham Hospital. Received for publication Feb. 15, 1917.

tests. When leaves are to be tested, they are macerated while green in n/100 sodium hydroxide for several days, then pressed through gauze and the extract is slowly evaporated over a water bath to dryness. Either a gummy mass or a dry crust results and this is used for the skin tests. As was stated in the previous papers, a drop of n/10 sodium hydroxide is placed on the skin, cut with the pollen or the extract of leaves, as the case may be, in order to dissolve them.

Skin reactions with the pollens are usually clear-cut urticarial wheals of varying sizes and only occasionally does an erythema without a wheal result. In the type of definite reactions which they produce the pollens closely resemble the alcoholic extracts of animal hair; these proteins give the largest reactions, probably because they are used in their original or natural state and have not been changed by manipulation in the preparation of their proteins as may be the case with the preparation of the food and the bacterial proteins.

The first part of the following description shows the relative frequency of reactions to the pollens of the Compositæ family and the second part shows how the same patient may react to the different members of the same biological family.

TABLE I.

The sensitization of patients with bronchial asthma to the pollens of the Compositæ family.

Pollen.	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Rag weed. (Ambrosia artemisifolia)	55	15	7	33
Goldenrod. (Solidago spi) . .	50	7	4	39
Daisy (white). (Chrysanthemum leucanthemum)	25	5	3	17
Sunflower. (Helianthus annuus)	7	3	1	3
Goldenglow. (Rudbeckia laciniata)	6	3	0	3

TABLE I. — *Continued.*

Patient.	Rag Weed.	Goldenrod.	Goldenglow.	Daisy.	Sunflower.
No. 1	4+	+	3+	2+	+
No. 2	O	O	O	2+	O
No. 3	+	+	+	+	+
No. 4	3+	±	+	2+	6+
No. 5	4+	+		+	
No. 6	3+	±			

In the first part of the table it is noted that in the same series of patients, twice as many are sensitive to rag weed as to goldenrod and that sensitization to the white daisy is very frequent. Since only those patients who were sensitive to both rag weed and goldenrod were tested with goldenglow and with sunflower we have a means of comparing the frequency of sensitization with each other and it is evident that the same patient does not give a positive skin reaction to all. The second part of the table illustrates the latter point better. One patient reacted positively to daisy and not with any other member of the Compositæ group which was tried, two patients reacted very strongly with rag weed but only doubtfully with goldenrod. The fourth patient reacted very strongly with sunflower (6+), gave a good positive with daisy and rag weed, a single plus with goldenglow and a very doubtful reaction with goldenrod. Only one patient reacted equally strongly with all pollens. It must be borne in mind that the whole concentrated pollens were used in these tests, therefore if a dilution of the pollen had been used many of the pollens would not have reacted at all. Consequently it would seem advisable to test with all pollens of the Compositæ family and to use the undiluted pollen.

TABLE II.

The sensitization of patients with bronchial asthma to the pollens of the Graminaceæ family.

Pollen.	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Red top. (Agrostis alba)	45	2	6	37
Timothy. (Phleum pratense)	45	5	5	35
Orchard grass. (Dactylis glomerata)	30	1	1	28
Corn. (Zea mays)	30	5	6	19

Patient.	Red Top.	Timothy.	Orchard Grass.	Corn.
No. 1	±	2+	O	±
No. 2	O	O	2+	2+
No. 3	O	+	O	+
No. 4	4+	4+	+	O
No. 5	6+	4+	O	O

In the same series of patients it is noted that more patients gave a positive reaction with timothy than with red top pollen and in a smaller number of cases in the same series only one patient gave a positive reaction with orchard grass, but five were positive with corn pollen. The large number of positive and doubtful reactions with corn pollen make us suspect that some irritating substance may be present in the corn pollen. In the second part of the table it is noted that the first case was positive with timothy and only doubtful or negative with the other pollens in this family. In Cases Nos. 4 and 5 very strongly positive reactions (4+ and 6+) were given with red top and timothy, but both cases were negative with corn and only one reacted with orchard grass. The second case gave strongly positive reactions with orchard grass and with corn, but failed to react at all with red top and with timothy and the third case reacted a single plus with timothy and corn, but gave no reaction with orchard grass

and red top. Again bearing in mind that whole pollen was used in these tests it would seem essential that the pollen of each of the widely-distributed members of the Graminaceæ family should be used in the skin tests and that whole pollen should be used rather than a dilution of it.

TABLE III.

The sensitization of patients with bronchial asthma to the pollens of miscellaneous families.

Pollen.	No. Patients Tested.	No. Patients Positive.	No. Patients Doubtful.	No. Patients Negative.
Rose. (Rosa rugosa)	20	0	2	18
Clover (white). (Trifolium incarnatum)	12	3	2	7
Lily. (Lilium superbum) . . .	7	0	0	7
Pear. (Pyrus)	11	0	0	11
Maple. (Acer saccharinum) .	9	0	0	9
Ash. (Fraxinus nigra)	6	1	0	5
Willow. (Salix spi)	20	2	0	18
Birch. (Betula alnifolia) . . .	6	0	0	6
Squash. (Cucurbita maxima) .	4	0	0	4
Sorrel. (Rumex acetocella) . .	8	0	0	8
Buttercup. (Ranuncular acris)	5	0	0	5
Pine. (Pinus anstriaca)	5	1	0	5

Patients were not tested with the pollens presented in this protocol as a matter of routine; they were used only when there seemed to be a reason for it, such as the patient's history or the home surroundings would suggest. It is noted that clover, ash, pine, and willow did give positive skin reactions in an occasional case.

It is not necessary to give a table illustrating that the same patient may be sensitive to the pollens of widely-separated families since this fact is well known. This paper has already brought out the necessity of testing with the whole pollen; naturally before an attempt is made to desensitize the patient different dilutions of the pollen should be tested in order to determine the correct amount with which to begin

desensitization. It is advisable to repeat at a later time all doubtful reactions and just previous to treatment they should be tested, since in the winter months cases may be negative to a particular pollen, but just before the season approaches the case may be positive.

Preparations of protein from the leaves of trees are used only in obscure cases and in those which seem to warrant it. One patient who had asthma only when at home and who reacted negatively with all of the pollens did give a positive reaction with poplar leaves and his house was surrounded with poplar trees. Another patient who reacted with willow leaves failed to react with willow pollen; this patient's house was surrounded with willow trees and treatment with willow pollen relieved all asthma. In obscure cases it would seem advisable to inspect the patient's surroundings and to make tests with extracts of the trees and other plants surrounding the home.

CONCLUSIONS.

In determining the cause of seasonal asthma, the patient should be tested with the pollen of all of the common plants rather than with one plant from each family; the whole pollen should be used in these tests rather than a dilution of the pollen.

Occasionally it is advisable to test with the leaves of trees and other plants since patients may give a positive reaction with the leaves and not with the pollen of that same plant.

STUDY XII.

Complement fixation and precipitin reactions with the serum of bronchial asthmatics who are sensitive to the proteins of wheat, horse dandruff, cat hair, and bacteria, using these proteins as antigens, and the cutaneous reaction as an index of sensitization.*

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In this study of bronchial asthma, complement fixation and precipitin reactions were done with the serum of patients who gave positive cutaneous reactions with the proteins of wheat, bacteria, horse dandruff, and cat hair. With the serum of those patients who were sensitive to the proteins in wheat, namely, gliadin, glutenin, globulin, leucosin, and natural proteose, these proteins were used as antigens; with the serum of those who were sensitive to the proteins in horse dandruff, namely, the alkali meta-protein, the peptone, and the coagulated protein, these were used as antigens; with those who were sensitive to the proteins in cat hair, namely, the alkali meta-protein and the peptone, these were used as antigens; and with those patients who were sensitive to the bacterial proteins these proteins were used as antigens. In several instances with patients who showed a multiple sensitization, all of the proteins to which they were sensitive were used as antigens, thus the same serum was tested with different proteins derived from very different sources and substances, and each type of protein antigen served as a control for the other protein antigen. Not only were complement fixation reactions done using the serum of patients with the antigen to which they were sensitive, but also using the serum of sensitive patients with other proteins

* This is the twelfth of a series of papers on the study of bronchial asthma made possible through a gift by Mr Charles F. Choate, Jr., to the Peter Bent Brigham Hospital. Received for publication Feb. 15, 1917.

to which they were not sensitive. Furthermore, reactions were done with the serum of normal and syphilitic patients, using all of the proteins described above as antigens; these serve as negative controls. Thus these complement fixation reactions using proteins as antigens would seem to be carefully and well controlled. That it is possible to obtain positive complement fixation reactions with proper serum, using the proteins of wheat as antigens, has been demonstrated by Lake, Osborne, and Wells.[1] They obtained positive fixation with high dilutions of wheat proteins as antigens with the serum of rabbits which had been injected with large amounts of the wheat proteins, and these fixations were specific for the particular protein employed.

By taking advantage of the condition of the patient at the time the blood was taken for the tests, this work presented exceptional opportunities. Patients who were sensitive to the wheat proteins ingested through the gastro-intestinal tract would be expected to have in their blood considerable antigen and few or no antibodies during asthmatic attacks; on a wheat-free diet the antigen would disappear from the blood and antibodies would be present; a further change in the blood content of antibodies might be made through the injection of small repeated doses of antigen for desensitization. The same factors could be controlled in the patients who are sensitive to the proteins in horse dandruff and cat hair by taking blood for the tests during, immediately after, and just before asthmatic attacks, and desensitizing injections with these proteins would be expected to alter the antibody content of the blood.

The cutaneous reaction as described in Studies III., IV., and V. was employed to determine the sensitiveness of the patient to the various proteins and the result of treatment in each case verified the cause of the asthmatic attacks. Therefore, as each case is presented in the protocols, the established cause of the asthmatic attacks is noted and also the proteins in the highest dilution to which the patient is sensitive and the complement fixation reactions with these proteins are given both before and after treatment. In this

way it is possible to show the relation between the cutaneous reaction or cell-sensitization and the complement fixation reaction, which shows the presence of antibodies in the blood, under various conditions. In a few instances precipitin tests were done and these will be recorded also.

The following antigens were used: Of the wheat proteins, leucosin, globulin, gliadin, glutenin, and natural proteose were used in the dilutions of 1–100, 1–1,000, 1–10,000, and 1–100,000. The dilutions were made with n/100 sodium hydroxide and the amount used in each test was .5 cubic centimeter of each dilution. Of the cat hair proteins, the peptone and the alkali meta-protein were used and of the horse dandruff proteins, the peptone, the alkali meta-protein, and the coagulated protein were used. These proteins were diluted in the same way and were used in the tests in the same amounts as was done with the wheat proteins. The bacterial antigens were made by growing large quantities of the organisms on large surfaces of agar, washing them off with normal saline, straining through gauze to remove chips of agar, and then centrifugalizing out the bacteria. The bacteria were again washed in saline, centrifugalized out, to the bacteria were added three or four volumes of normal saline and this mixture was shaken in a shaker for forty-eight hours, after which the bacteria were centrifugalized out and the fluid was used as antigen. In the tests three different amounts of this antigen, namely .5, .25, and .1 cubic centimeter, were used with each serum. Staphylococcus pyogenes aureus was the only organism from which antigens were made.

The technic used in these complement fixation reactions was the same as that of the Wassermann reaction. Each day the reactions were done the hemolytic system was standardized. In the tests in each tube was placed .1 or .2 cubic centimeter of the patient's serum previously inactivated, two units of complement diluted with saline to make .5 cubic centimeter antigen, .5 cubic centimeter of each dilution, as mentioned above, and normal saline .4 cubic centimeter, thus making the total volume of each tube 1.5 cubic

centimeters. The tubes were placed in a water bath at 37.5° C. for one hour, then one cubic centimeter of sensitized sheep cells was added to each tube and again the tubes were placed in the water bath for one hour, after which the results were read. The proteins in dilutions of 1–100 became anti-complementary after standing for a few weeks, and coincident with this there was a precipitation of the protein; other dilutions of the proteins, however, showed no precipitation and did not become anti-complementary.

The patients on whom complement fixation reactions were done will be grouped into protocols according to the type of antigen used and to the type of fixation given by them. That is, one protocol will contain the patients who give a *negative* fixation with the horse dandruff proteins, another protocol will contain the patients who give a *positive* fixation with the horse dandruff proteins and so on with the cat hair, wheat, and bacterial proteins. In order to save space in each protocol this opportunity is taken to mention the known negative controls for the whole paper. Each time complement fixation reactions were done with the proteins of horse dandruff, cat hair, and wheat the serum of normal and of syphilitic patients was used as known negative serum controls; antigen controls will be mentioned as they occur. With the bacterial cases, similar negative serum controls were used and antigen controls were made from S. pyogenes albus and from M. catarrhalis and M. tetragenous; none of these gave positive fixation with the cases studied.

Protocol I.

Negative Complement Fixation Reactions with the Horse Dandruff Proteins as Antigens.

D. G., a woman aged 22, has had asthma for eighteen months and associates some of the attacks with horses. The cutaneous tests with alkali meta-protein in a dilution of 1–100,000 = +, with peptone 1–100 = O, and with coagulated protein 1–1,000 = +. Complement-fixation tests were done two days after an attack of asthma, and were negative with these three proteins in dilutions from 1–100 to 1–1,000,000 inclusive. The patient was treated subcutaneously each week with gradually increasing amounts of the alkali meta-protein for four months, during which time she had no asthmatic attacks. Complement fixation tests were again done and found to be negative as before, the cutaneous reactions, however, had

greatly diminished (100 times weaker), and the alkali meta-protein now gave a negative reaction in a dilution of 1–1,000, and the other two proteins were positive only with the concentrated protein.

F. L. D., a man aged 48, has had running of the nose, watering of the eyes, sneezing with an occasional attack of asthma for twenty-five years, only when riding behind horses. Cutaneous reactions with the alkali meta-protein, the peptone, and the coagulated protein, each in dilutions of 1–100,000, were positive. Complement fixation reactions with these proteins in dilutions from 1–100 to 1–1,000,000 were negative. Preceding these tests the patient had had no asthma for several months.

L. T., a school boy aged 13, has had asthma since birth and had recognized that some of the attacks were associated with horses. Cutaneous reactions with the alkali meta-protein and with the peptone, each in dilutions of 1–10,000, were positive and the coagulated protein 1–100,000 was positive. Complement fixation reactions were negative with these proteins in dilutions from 1–100 to 1–1,000,000. The patient had had asthma, after fondling a horse, one week previous to these tests. This case also occurs in a later protocol with the cat hair protein fixations.

J. T. S., a man aged 48, who grooms and drives a six-horse team has had asthma for six years. After three months' treatment with subcutaneous injections of horse dandruff peptone, the cutaneous reactions became negative, he had been free from asthma for a month, and the complement fixation reactions were entirely negative.

S. H. G., aged 42, the wife of a hostler, has had asthma for sixteen years and associates some of the attacks with horses. The cutaneous reactions with the alkali meta-protein, the peptone, and the coagulated protein each in dilutions of 1–10,000 were positive. Complement fixation reactions with these proteins were negative in all dilutions from 1–100 to 1–1,000,000 inclusive. This patient had an attack of asthma the previous week.

M. D., aged 38, a housewife, has had asthma for ten years, and associates attacks with cats. Cutaneous reactions with the horse dandruff proteins each in a dilution of 1–10,000 were positive. Complement fixation reactions with these proteins were negative in all dilutions. This case occurs in a later protocol with the positive cat hair protein complement fixations.

G. B., a girl aged 17, has had asthma since eighteen months of age with no association with horses. Cutaneous reactions with the alkali meta-protein, and with the coagulated protein each in dilutions of 1–10,000, and with the peptone in a dilution of 1–1,000 were positive. Complement fixation reactions with these proteins in all dilutions from 1–100 to 1–1,000,000 were negative. This case occurs in a later protocol with the positive wheat protein complement fixations.

G. V. Y., aged 28, has had asthma for seven years and associates some attacks with cats. Cutaneous reactions and complement fixation reactions with the horse dandruff proteins were negative. This case occurs with the positive cat hair protein complement fixation reactions.

The above protocol presents eight patients who gave negative complement fixation reactions, using the horse dandruff proteins as antigens. Five of these patients knew that some or all of their asthmatic attacks were caused by horses, and these same patients together with two of the others gave definitely positive cutaneous reactions with high dilutions of the three proteins found in horse dandruff. A discussion of each case follows:

The first case (D. G.), who gave a positive cutaneous reaction with the alkali meta-protein in a dilution of 1–100,000 and with the coagulated protein in a dilution of 1–1,000, knew that horses were a cause of her asthma. Furthermore, an attack of asthma was produced in this case by a subcutaneous injection of the alkali meta-protein, and during the past four months while under treatment with desensitizing doses of this protein she has had no asthma. Therefore this patient would seem to have definite horse asthma. Nevertheless complement fixation reactions were negative two days after an attack of asthma, at which time one would expect antibodies to be present in the blood. After a period of four months' treatment with desensitizing doses of the alkali meta-protein, complement fixation reactions were still negative although the cutaneous reaction was one hundred times weaker.

The second case (F. L. D.), which was a clear-cut one of horse hay fever and asthma, gave positive cutaneous reactions with the three horse dandruff proteins each in a dilution of 1–100,000. Complement fixation reactions were negative and the patient had not been exposed to horses and had had no symptoms for the preceding several months.

The third case (L. T.) recognized horses as a cause of his asthma, gave positive cutaneous reactions with all three of the proteins in high dilutions and had no asthma for two months during treatment with desensitizing doses of the

coagulated protein. Complement fixation reactions were negative with all dilutions of the three proteins, even though a week previous to the tests the patient had had several days of asthma following patting and fondling of a horse.

The fourth case (J. T. S.) was definitely a case of horse asthma and was relieved of all asthma by desensitizing doses of the horse dandruff peptone, so that he was able to groom his six-horse team without any trouble. When treatment was discontinued cutaneous reactions and complement fixation reactions were negative with all dilutions of each protein.

The fifth case (S. H. G.), who is the wife of a hostler and who is so sure that her asthma is due to horses that precautions are taken at home against the entrance of horse hair and dandruff into the house, gave positive cutaneous reactions with the three horse dandruff proteins, each in a dilution of 1–10,000. The week following an asthmatic attack complement fixation reactions were negative with all dilutions of each of the proteins.

The sixth case (M. D.), who associated her asthma with cats and who gave positive cutaneous reactions with the cat hair proteins in a dilution of 1–100,000, gave positive cutaneous reactions with each horse dandruff protein in a dilution of 1–10,000. Complement fixation reactions were negative with all dilutions of each of the three horse dandruff proteins, but they were positive with the cat hair proteins. This case will be discussed in Protocol III. with the positive fixations with the cat hair proteins.

The seventh case (G. B.), who did not associate asthma with horses, gave positive cutaneous reactions with the horse dandruff proteins in high dilutions and gave negative complement fixation reactions with all dilutions of these proteins. This case will be discussed in Protocol V. with positive complement fixation reactions with the wheat proteins as antigens.

The last case (G. V. Y.), who has asthma from cats, gave negative cutaneous and complement fixation reactions with all dilutions of the three horse dandruff proteins. This case will be discussed in Protocol III. with the positive fixations with cat hair proteins.

Therefore from these cases it is seen that some patients, whose asthma is definitely caused by the horse and who are definitely sensitive to very minute amounts of the horse dandruff proteins, fail to give positive complement fixation reactions using these proteins in a wide range of dilutions as antigens. Furthermore, complement fixation reactions were negative when the blood was taken both soon after and a long time after an asthmatic attack which was caused by the same antigens which were used in the tests. Complement fixation reactions remained negative after a long series of desensitizing doses with the antigen, even though this treatment was sufficient to protect the patient against asthma and to decrease the positiveness of the cutaneous reactions one hundred times or to render them negative. The last three cases were tested against several antigens. At the same time that these patients gave negative complement fixation reactions with the horse dandruff proteins, they did give positive complement fixations with the cat hair or wheat proteins, which were proven to be the cause of their asthma and to which the patients were sensitive, so that these reactions were doubly controlled.

Protocol II.

Positive Complement Fixation Reactions with the Horse Dandruff Proteins as Antigens.

C. N. E., a man aged 25, has had asthma for twenty years and associates some of the attacks with horses. Cutaneous reactions with the alkali meta-protein and with the peptone in dilutions of 1–10,000 and with the coagulated protein in a dilution of 1–100,000 were positive. Complement fixation reactions were positive with the coagulated protein in dilutions from 1–100 to 1–100,000, with the alkali meta-protein and with the peptone they were negative in all dilutions. Precipitin tests were negative with these proteins in all dilutions using .1 cubic centimeter of serum and .5 cubic centimeter of antigen; all precipitin reactions were read after standing two hours in the water bath at 38° C , and again after twenty hours in the ice-box. The patient was treated subcutaneously with two injections of coagulated protein in a dilution of 1–1,000,000, with seven doses in a dilution of 1–100,000 and once with a dilution of 1–10,000. Two months after the last treatment complement fixation tests were repeated and found to be negative with all dilutions of all three proteins.

D. A. G., a boy aged 19, has had asthma since three years of age; some of these attacks have been associated with horses. Cutaneous reactions

with the three proteins of horse dandruff were positive in dilutions of 1–10,000. Complement fixation tests were positive with the coagulated protein in dilutions of 1–100 to 1–100,000 inclusive, with the alkali meta-protein in dilutions of 1–100 and 1–1,000, but were negative with all dilutions of the peptone. The patient was treated subcutaneously with three doses of all three proteins in a dilution of 1–100,000 and four doses in a dilution of 1–10,000. Complement fixation reactions were repeated and were negative with all dilutions of all these proteins. This case is presented later on with the negative complement fixation reactions using the wheat proteins as antigens.

J. M., a school boy aged 9, has had asthma for six years and had not recognized horses as a cause. Cutaneous reactions with the alkali meta-protein in a dilution of 1–10,000 and with the coagulated protein in a dilution of 1–1,000 were positive; the peptone was negative in a dilution of 1–100. Complement fixation reactions were positive with the alkali meta-protein and with the peptone in dilutions from 1–100 to 1–10,000 inclusive, and with the coagulated protein from 1–100 to 1–100,000 inclusive. Precipitin tests were positive with the peptone in dilutions of 1–100 and 1–1,000, but were negative with the alkali meta-protein and with the coagulated protein. In these tests .2 cubic centimeter of serum and .5 cubic centimeter of antigen were used.

In Protocol II. are presented three patients who gave positive complement fixation reactions with one or more of the horse dandruff proteins. Two of the cases associated some of their asthmatic attacks with horses and all three patients gave positive cutaneous reactions with the three proteins from horse dandruff.

The first case (C. N. E.), which gave positive cutaneous reactions with the alkali meta-protein and with the peptone each in a dilution of 1–10,000, failed to give positive complement fixation reactions with these proteins in any dilution. The coagulated protein, however, gave both positive cutaneous and complement fixation reactions in a dilution as high as 1–100,000. Precipitin tests were not given with any dilution of the three proteins. Complement fixation reactions with the coagulated protein were rendered negative by ten desensitizing doses with this protein; the majority of these doses were with a dilution of 1–100,000.

The second case (D. A. G.), which gave positive cutaneous reactions with all three proteins in the same dilution,

namely, 1–10,000, gave positive complement fixation reactions with the coagulated protein in a dilution as high as 1–100,000, but with the alkali meta-protein in a dilution only as high as 1–1,000, and with the peptone no fixation was given in any dilution. At the same time these tests were done, negative complement fixations were obtained with the wheat proteins, and positive fixations were obtained with the cat hair proteins using the same specimen of serum with all the different antigens. Seven desensitizing doses with a mixture of all three proteins in dilutions of 1–100,000 and 1–10,000 rendered the complement fixation reactions negative with all three proteins in all dilutions.

The third case (J. M.), the only one who had not associated his asthma with horses, gave positive, cutaneous, and complement fixation reactions with the alkali meta-protein in the same dilution, namely, 1–10,000, but with the coagulated protein complement fixation reactions were positive in a dilution one hundred times greater than was given by the cutaneous reaction and the peptone, which gave a negative cutaneous reaction in a dilution of 1–100, did give a positive complement fixation reaction in a dilution as high as 1–10,000. Furthermore, precipitin tests were positive with the peptone in dilutions of 1–100 and 1–1,000, but they were negative with the other two proteins, which gave stronger cutaneous and complement fixation reactions.

In these three cases it is impossible to correlate the results of the cutaneous, complement fixation, and precipitin reactions. The first case was sensitive to all three proteins but gave positive complement fixations with only one protein and failed to give precipitin tests with any. The second case, which was sensitive to all three proteins in the same dilution, gave positive complement fixation reactions with the coagulated protein in a higher dilution and with the alkali meta-protein in a lower dilution; with the peptone no fixation was given. In the third case peptone gave a negative cutaneous reaction, but gave a positive complement fixation and precipitin reaction. The other two proteins gave positive cutaneous and complement fixation reactions, but negative

precipitin tests. A very small number of treatments with these proteins in a high dilution rendered negative previously positive complement fixation reactions.

Protocol III.

Positive Complement Fixation Reactions with the Cat Hair Proteins as Antigens.

G. V. Y., housewife aged 28, has had asthma for seven years and associates some attacks with cats. Cutaneous reactions with cat hair alkali meta-protein and with peptone were positive in a dilution of 1–1,000,000 each. Complement fixation reactions with these two proteins were positive in all dilutions from 1–100 to 1–10,000,000 inclusive. Precipitin tests using .2 cubic centimeter of serum and .5 cubic centimeter of antigen were positive the next morning only in dilutions of 1–100 with each protein. The patient was treated subcutaneously with five doses of the cat hair alkali meta-protein in a dilution of 1–1,000,000 and with ten doses in a dilution of 1–100,000. After these treatments the cutaneous reactions had decreased one hundred times; each protein was now positive in 1–10,000 dilution and the complement fixation reactions were negative with all dilutions of each protein. The patient has been free from asthma for over five months. (This case has been presented in Protocol I.)

M. D., a woman aged 38, has had asthma for ten years and has associated many attacks with cats. Cutaneous reactions with cat hair alkali meta-protein and peptone were positive in dilutions of 1–100,000. Complement fixation reactions with the protein were positive in dilutions of 1–100 and 1–1,000, but were negative with higher dilutions, and they were negative with all dilutions of the peptone.

D. A. G., a boy aged 19, has had asthma for sixteen years. Cutaneous reactions were positive with the alkali meta-protein and with the peptone each in a dilution of 1–1,000. Complement fixation reactions were positive with alkali meta-protein in dilutions of 1–100 and 1–1,000, but were negative with all dilutions of the peptone. This case is presented also in Protocol II, with positive horse dandruff protein fixations and in Protocol IV. with the negative wheat protein fixations.

In Protocol III. are presented three cases, two of which are definitely cat asthmatics. The first case (G. V. Y.) is very interesting in that it gave positive cutaneous reactions with each cat hair protein in a dilution of 1–1,000,000 and positive complement fixation reactions with each protein in a dilution of 1–10,000,000, but gave positive precipitin reactions with each protein only in a dilution of 1–100. At the same time that these tests were done with this patient, complement fixation reactions were negative with the three horse

dandruff proteins so that the positive reactions with the cat hair proteins were well controlled. Subcutaneous injections with the alkali meta-protein alone changed the complement fixation reactions with both proteins from positive to negative and diminished the positiveness of the cutaneous reactions with each protein one hundred times.

The second case, which gave positive cutaneous reactions with each protein in a dilution of 1–100,000, gave positive complement fixation reactions with the alkali meta-protein only as high as 1–1,000 and gave negative fixation reactions with the peptone. The third case, which was sensitive to each protein in a dilution of 1–1,000, gave positive fixation with the protein in that dilution, but negative fixation with the peptone. At the same time these reactions were done two of the horse dandruff proteins gave positive fixation and all of the wheat proteins gave negative fixations.

Therefore, in general, the results presented in this protocol duplicate those presented in Protocol II. so that it is as impossible to correlate the cutaneous, complement fixation, and precipitin reactions. Two of the three cases failed to give positive fixation with the peptone when the other protein did give fixation. The other case gave positive fixation with a higher dilution than was required to give a positive cutaneous reaction and this same case gave a positive precipitin reaction only in a concentrated solution. Treatment with one protein antigen rendered positive fixation negative with both proteins.

Protocol IV.

Negative Complement Fixation Reactions with the Wheat Proteins as Antigens.

H. M., a housewife aged 37, has watering of the eyes, running of the nose, and sneezing only when she mixes bread. Cutaneous reactions with natural proteose and with leucosin were positive in a dilution of 1–1,000, with artificial proteose and with globulin the reactions were ++ with the concentrated protein, with gliadin and glutenin they were a single plus with the undiluted protein, whole wheat was positive in a dilution of 1–500 and all the cereal proteins were more or less positive. Complement fixation reactions were negative using as antigen each protein in dilutions of 1–100 to 1–100,000 inclusive.

J. D , a baker aged 40, has asthma only when working with flour. Cutaneous reactions with natural proteose, glutenin, gliadin, and leucosin each in a dilution of 1–100 were positive, globulin in a dilution of 1–1,000 was positive, the concentrated artificial proteose gave a doubtful reaction, the concentrated whole wheat and wheat bread proteins were strongly positive. Complement fixation reactions with each of these proteins as antigens were negative with all dilutions from 1–100 to 1–100,000 inclusive. Omitting all wheat from the diet relieved the patient from all asthma even while baking.

W. P. H., a man aged 25, has had asthma for ten years. Cutaneous reactions with globulin and glutenin were a +, with gliadin and leucosin they were a 2+, with whole wheat a 2+, but with the artificial and natural proteose were negative; all cereals were more or less positive. Complement fixation reactions were negative using as antigens all the wheat proteins. On a wheat-free diet all asthma ceased.

D. A. G., a boy aged 19, has had asthma since three years of age. Cutaneous reactions with glutenin and gliadin were +, with natural proteose a 2+, with globulin a 3+ and with whole wheat a +; the artificial proteose and the wheat bread protein gave doubtful reactions. After two weeks of a wheat-free diet during which the patient was free from asthma, complement fixation reactions were done and were negative with all the wheat proteins in all dilutions from 1–100 to 1–100,000 inclusive. This patient is also presented in Protocol II. with the positive complement fixation reactions with the horse dandruff proteins and in Protocol III. with the positive complement fixation reactions with the cat hair proteins.

C. J., a man aged 30, has had asthma for many years and all attacks were associated either with horses or with indigestion. Cutaneous reactions were positive with glutenin, globulin, leucosin, and with natural proteose, each in a dilution of 1–100; the concentrated proteins, gliadin and artificial proteose, were negative. Complement fixation reactions with all of these proteins in dilutions of 1–100 to 1–100,000 inclusive were negative. After the patient had been on a wheat-free diet for three weeks complement fixation reactions were repeated and were found to be negative as before; this time duplicate tests were done using .1 cubic centimeter of serum with one set and .2 cubic centimeter with the second set of tests.

In Protocol IV. are presented five cases which gave positive cutaneous and negative complement fixation reactions with the different wheat proteins as antigens and in four of these cases wheat proteins have proved to be a cause of the asthma.

The first case (H. M.), who has hay fever only when mixing flour, gave positive cutaneous reactions with each wheat protein, including leucosin and natural proteose, in a dilution of 1-1,000; whole wheat was positive in a dilution of 1-500. Nevertheless complement fixation reactions were negative with each protein.

The next case (J. D.), who is a baker, has asthma only when working in the bakeshop. Although this patient was sensitive to the wheat proteins in a dilution of 1-100 complement fixation reactions were negative; the blood for these tests was taken when the patient was on a wheat diet and was having asthma. Since all wheezing ceased when the patient omitted wheat from his diet and since a subcutaneous injection of wheat protein was followed by an attack of asthma, wheat would seem to be the cause of asthma in this case.

The third case (W. P. H.), which was sensitive to most of the individual proteins in wheat and to whole wheat protein, gave negative complement fixation reactions; the blood for these tests was taken when the patient was free from wheezing. On a wheat-free diet this patient had no asthma and a subcutaneous injection of wheat protein was followed by asthma. During a continuous wheat-free diet the patient has been free from asthma.

The fourth case (D. A. G.), who was sensitive to all the wheat proteins, was on a wheat-free diet for two weeks before the blood was taken, but the complement fixation reactions were negative with the wheat proteins, at the same time, however, they were positive with the horse dandruff and cat hair proteins. For the past two months this patient has been on a wheat-free diet with weekly desensitizing doses of whole wheat protein. During this time the patient has been free from wheezing, except for one day when he ate spaghetti.

The last case (C. J.), which was very sensitive to the wheat proteins, gave negative complement fixation reactions on two occasions; the first time while on a wheat diet and the second time while on a wheat-free diet. It is impossible to judge at the present time what part wheat plays as the cause

of asthma in this case since horses are known to be a cause of his asthma.

Therefore the conclusions from these cases are similar to those from the cases occurring in Protocol I.; the only difference is that different proteins were used as antigens. Some patients who definitely have asthma from the wheat proteins and who are very sensitive to these proteins do not give positive complement fixation reactions when these proteins are used as antigens. Complement fixation reactions were negative whether the blood was taken when the patient was on a wheat diet and was wheezing or when he was on a wheat-free diet and not wheezing; in the latter case one would expect to find antibodies in the serum. In one case complement fixation reactions were done with several types of antigens; the wheat protein antigens gave no fixation, but the horse dandruff and the cat hair protein antigens gave positive fixation.

Protocol V.

Positive Complement Fixation Reactions with the Wheat Proteins as Antigens.

G. B , a girl aged 17, has had asthma since eighteen months old. Cutaneous reactions with globulin, glutenin, and leucosin were each a 2 +, with gliadin a +, but with each proteose they were negative. Complement fixation reactions were positive with globulin in a dilution of 1–100,000 and with glutenin, gliadin, and natural proteose in dilutions of 1–10,000 and 1–1,000 with each protein, but they were negative with lower dilutions of these and with leucosin they were negative with all dilutions. Wheat was omitted from the diet and the asthma ceased, after eating wheat asthma returned again promptly, again wheat was omitted and asthma stopped, a third time wheat was eaten and asthma returned promptly, and a third time wheat was omitted from the diet and asthma stopped as before. This case occurs in Protocol I. with the negative horse dandruff protein fixations.

M. S., housewife aged 31, has had asthma for fifteen years. Cutaneous reactions with all of the wheat proteins were weakly positive. Complement fixation reactions were positive with gliadin, globulin, glutenin, and natural proteose in dilutions of 1–10,000 and 1–100,000 of each protein, but they were negative with lower dilutions, and with leucosin they were negative with all dilutions. During treatment with subcutaneous injections of whole wheat protein in a dilution of 1–500 and 1–100 all asthma ceased and complement fixation reactions became entirely negative with all proteins in all dilutions.

F. G., housewife aged 42, has had asthma for over twenty years. Cutaneous reactions with glutenin were negative, with gliadin, globulin, and natural proteose they were slightly positive and with leucosin they were 3+ and in a dilution of 1–100 they were positive. Complement fixation reactions were positive with globulin in a dilution of 1–100,000 and with gliadin, glutenin, and natural proteose each in dilutions of 1–10,000 and 1–100,000, but were negative with lower dilutions and with leucosin they were negative with all dilutions. The patient was treated with subcutaneous injections of leucosin in gradually increasing doses of a dilution of 1–1,000 without any improvement in the asthma. The patient was then put on a wheat-free diet for three weeks without any improvement in the asthma, therefore ruling out wheat proteins as a cause of the asthma. Complement fixation reactions were repeated at this time and were found to be negative with all proteins in all dilutions, using both .1 and .2 cubic centimeter of serum.

G. B., a baker aged 52, has had asthma for nine years. Cutaneous reactions were slightly positive with leucosin, glutenin, gliadin, and artificial proteose, were strongly positive with globulin and with natural proteose in a dilution of 1–100; all cereal proteins were positive, including corn in a dilution of 1–100. Complement fixation reactions were positive with globulin, gliadin, glutenin, and natural proteose in all dilutions from 1–100 to 1–100,000 inclusive, but with leucosin they were negative with all dilutions. The patient was treated subcutaneously with corn protein and with globulin and natural proteose in dilutions of 1–1,000 and 1–100, five times without any benefit. Complement fixation reactions had now become negative with all proteins in all dilutions. The patient was then put on a wheat-free diet for three weeks with no improvement in the asthma, therefore wheat proteins probably had little or no part in the cause of the asthma.

In Protocol V. are presented four cases which gave positive cutaneous reactions with some or all of the wheat proteins and positive complement fixation reactions with the majority of these proteins. In one of these cases wheat was proved to be the cause of asthma, in another wheat seemed to be the cause since treatment with whole wheat relieved the asthma, but in the remaining two cases wheat apparently played little or no part in the cause of asthma at this time, although it may have been the primary cause at an earlier date; now chronic bronchitis would seem to be the chief cause.

In the first case (G. B.) wheat is regarded as the cause of asthma since during three intervals while on a wheat-free diet

asthma and wheezing ceased and during three different periods of wheat diet asthma returned. The patient gave positive cutaneous reactions with all of the wheat proteins with the exception of both proteoses, and with the exception of leucosin complement fixation reactions were positive in high dilutions with all of the wheat proteins, including natural proteose to which the patient was not sensitive. When these tests were done the patient was having asthma and was on a wheat diet. Thus this case gave positive cutaneous and negative fixation reactions with leucosin and negative cutaneous and positive fixation reactions with natural proteose; the remaining wheat proteins gave both positive cutaneous and fixation reactions in high dilutions, but no fixation was given with low dilutions of these proteins as one would expect.

The second case (M. S.), which was relieved of asthma by desensitizing injections of whole wheat protein, gave weakly positive cutaneous reactions with each wheat protein and positive complement fixation reactions in high dilutions only of each protein with the exception of leucosin. Following treatment with desensitizing doses of whole wheat protein, complement fixation reactions became negative.

In the third case (F. G.) leucosin was the only wheat protein which gave positive cutaneous reactions, but all of the other wheat proteins gave positive complement fixation reactions. Following desensitizing doses with leucosin, together with a wheat-free diet for a period of three weeks, complement fixation reactions were negative with all of the wheat proteins. Since no improvement followed this treatment and since the patient was no worse when she returned to a wheat diet it would seem that wheat played no part in the cause of asthma in this case.

The last case (G. B.) is similar to the preceding one. Cutaneous reactions were positive only with wheat globulin and natural proteose; complement fixation reactions, however, were positive with all of the wheat proteins with the exception of leucosin. Since the patient was also very sensitive to corn protein desensitizing doses were given both with

corn protein and with the two wheat proteins to which the patient was sensitive. Following this treatment complement fixation reactions were negative with all of the wheat proteins. Since the patient did not improve under this treatment or while on a wheat-free diet, and since returning to a wheat diet made the patient no worse, wheat would seem to play no part in the cause of asthma in this case.

It is as impossible to correlate positive cutaneous and complement fixation reactions with the wheat proteins as it was with the horse dandruff and cat hair proteins. No case gave positive complement fixation reactions with wheat leucosin although positive cutaneous reactions were given by it, and one patient who was sensitive to leucosin alone gave positive complement fixation reactions with all of the other wheat proteins and not with leucosin. It mattered not whether the patient was weakly or strongly sensitive to the wheat proteins, complement fixation reactions were positive in the same dilutions in either case. A very few desensitizing doses with the antigen changed a previously positive fixation to a negative one, as was the case with the horse dandruff proteins. Positive fixation with high dilutions of antigen and negative fixation with more concentrated dilutions of the same antigen are difficult to explain.

Protocol VI.

Complement Fixation Reactions with the Antigen made from Staphylococcus Pyogenes Aureus.

M. S., a woman aged 31, has had asthma for nineteen years. Cutaneous reactions with the protein from S. pyogenes aureus were positive. Complement fixation reactions with the antigen made from this organism were negative with .1 cubic centimeter of serum and .5, .25, and .1 cubic centimeter of antigen. Precipitin reactions were negative with .1 cubic centimeter of serum and .4, .6, and .8 cubic centimeter respectively of antigen. During treatment with vaccines made with S. pyogenes aureus, all asthma ceased and remained absent for over six months after the vaccines were discontinued; the cutaneous reaction also became negative.

J. H. N., a man aged 29, has had asthma for five years. Cutaneous reaction with S. pyogenes aureus protein was positive. Complement fixation reactions with .1 cubic centimeter of serum and .5, .25, and .1 cubic centimeter of antigen were negative. The precipitin reaction was

positive with .1 cubic centimeter of serum and .6 cubic centimeter of antigen. During desensitization with the protein of this organism, the asthma gradually diminished in frequency and intensity, and the precipitin reaction became negative.

A. D., a woman aged 30, has had asthma for eighteen months. Cutaneous reactions with S. pyogenes aureus protein were positive. Complement fixation reactions using .1 cubic centimeter of serum and .1 cubic centimeter of antigen were positive. The precipitin reaction was positive at the end of two hours using .1 cubic centimeter of serum and .6 cubic centimeter of antigen. After four desensitizing doses with the protein from this organism, the complement fixation reaction became negative.

D. J. H., a man aged 28, has had asthma for sixteen years. Cutaneous reactions with S. pyogenes aureus protein were slightly positive. Complement fixation reactions were positive with .1 cubic centimeter of serum and .1 cubic centimeter of antigen. This patient also was sensitive to and had asthma from horses.

M. C., a woman aged 51, had asthma for three years. Cutaneous reactions were positive with S. pyogenes aureus protein. Complement fixation reactions were negative with this antigen. Precipitin reactions were positive with .1 cubic centimeter of serum and .6 and .8 cubic centimeter of antigen.

B. K., a woman aged 23, had asthma for one year. Cutaneous reactions with S. pyogenes aureus protein were positive. Complement fixation reactions with this antigen were positive using .1 cubic centimeter of serum and .1 cubic centimeter of antigen. Precipitin reactions were positive using .1 cubic centimeter of serum with .4 cubic centimeter of antigen. On repeating the complement fixation reactions two months later, with no treatment in the meantime, they were still positive.

In Protocol VI. are presented six cases which gave positive cutaneous reactions with S. pyogenes protein. Since three of the patients gave positive complement fixation reactions, and the other three gave negative complement fixation reactions with S. pyogenes antigen, each group of three cases will be discussed separately, as was done in the preceding protocols.

The following cases, M. S., J. H. N., and M. C., gave positive cutaneous reactions and negative complement fixation reactions with S. pyogenes aureus antigen. Precipitin tests were negative in the Case M. S., but were positive

in the other two cases. Following desensitizing doses with S. pyogenes aureus protein in Case J. H. N., the precipitin reaction became negative. Since Case M. S. was relieved of asthma by S. pyogenes aureus vaccine and Case J. H. N. was greatly improved during treatment with desensitizing doses of S. pyogenes aureus protein, the asthma in these two cases would seem to be caused by S. pyogenes aureus.

The Cases A. D., D. J. H., and B. K. gave positive cutaneous and positive complement fixation reactions with S. pyogenes aureus antigen and precipitin tests were positive in the two cases in which they were tried. Following treatment with desensitizing doses of S. pyogenes aureus protein in Case A. D., complement fixation reactions became negative; in Case B. K., which was not treated, complement fixation reactions were still positive two months later.

Therefore in this series of cases it is impossible to correlate cutaneous, complement fixation, and precipitin reactions, as was the case in the preceding protocols. Either, any two, or all three of these reactions were positive in the individual cases. As occurred in the preceding protocols complement fixation and precipitin reactions became negative following treatment with the protein.

Summary. — Complement fixation reactions were done using the three proteins of horse dandruff as antigens with the serum of eleven patients with bronchial asthma. Eight of these patients gave negative fixation and three gave positive fixation. Of the eight who gave negative fixation, seven gave positive cutaneous reactions with one or more of the three horse dandruff proteins in a dilution of 1–10,000 or higher, and in four of these patients horse dandruff proteins were proved to be the cause of the asthma. These complement fixation reactions were negative whether the blood was taken shortly after or a long time after an attack of asthma which was caused by the antigen, and these reactions remained negative following a short or a long series of desensitizing injections with the antigen, even though these injections were sufficient to prevent the patient from having asthma, and

were sufficient to diminish the positiveness of the cutaneous reaction one hundred times or to make it negative. At the same time that three of these patients gave negative fixations with the three horse dandruff proteins, these patients did give positive fixations either with the proteins from cat hair or from wheat. In the three cases which gave positive fixation with one or more of the horse dandruff proteins, it is impossible to correlate the results of the cutaneous, complement fixation, and precipitin reactions. One case gave positive cutaneous reactions with all three proteins from horse dandruff, but gave positive fixation with only one of them and gave a negative precipitin reaction with all. Another patient gave positive cutaneous reactions with each of the proteins in a dilution of 1–10,000, and gave positive complement fixation with the coagulated protein in a higher dilution, and with the alkali meta-protein in a lower dilution than was positive with the cutaneous reaction; the peptone gave no fixation. In the remaining case, horse dandruff peptone gave no cutaneous reaction, but it did give positive complement fixation and precipitin reactions. The other two proteins gave positive cutaneous and fixation reactions in this case but the precipitin reaction was negative. In the two cases which were treated with desensitizing injections of the proteins or antigens, complement fixation reactions were changed from positive to negative.

In three patients complement fixation reactions were positive with one or both of the proteins from cat hair, and two of the three patients were definitely cat asthmatics. With these cat hair proteins, correlation of the cutaneous, complement fixation, and precipitin reactions was as difficult as it was with the horse dandruff proteins. One patient gave positive cutaneous reactions with each protein in a dilution of 1–1,000,000 and positive fixation in a dilution of 1–10,000,000 and positive precipitin reactions in a dilution of only 1–100. Treatment with desensitizing injections of the alkali meta-protein alone changed the previously positive fixation with both proteins to negative with both proteins, and diminished the positiveness of the cutaneous

reaction with both proteins one hundred times. The other two patients gave positive complement fixation reactions with the alkali meta-protein, but negative fixation with the peptone, although each protein gave positive cutaneous reactions in high dilutions.

Complement fixation reactions were done with the serum of nine asthmatics using the individual wheat proteins as antigens, and of these nine patients, five gave negative fixation and four gave positive fixation. Of the five patients who gave negative complement fixation reactions it may be stated that some, who definitely have asthma from the wheat proteins and who are very sensitive to these proteins, do not give positive fixation with these proteins as antigens. Complement fixation reactions were negative whether the blood was taken when the patient was on a wheat diet and having asthma or when he was on a wheat-free diet and free from asthma. Of the four patients who gave positive complement fixation reactions with one or more of the wheat proteins, it may be stated that no case gave positive fixation with wheat leucosin, although positive cutaneous reactions were given by it, and one patient who was sensitive to leucosin alone gave positive fixation with all of the other wheat proteins and not with leucosin. It mattered not whether the patient was weakly or strongly sensitive to the wheat proteins, complement fixation reactions were positive in the same high dilutions in either case. A very few desensitizing injections with one wheat antigen changed a previously positive fixation to a negative one, as was the case with the horse dandruff and cat hair proteins. Positive fixation with high dilutions of antigen and negative fixation with more concentrated dilutions of the same antigen with the same serum are difficult to explain.

Six patients, who gave positive cutaneous reactions with S. pyogenes aureus protein, gave similar complement fixation and precipitin reactions as was given by the preceding cases. Three of these gave positive complement fixation and precipitin reactions and three gave negative complement fixation reactions, although two of the latter gave positive precipitin

reactions. Following desensitizing injections with the protein, positive complement fixation and precipitin reactions were changed to negative.

Therefore in a general way the results given by complement fixation and precipitin reactions were similar with each type of protein. Cases who are very sensitive to these proteins and whose asthma is definitely caused by these proteins may or may not give positive complement fixation and precipitin reactions, in fact in the majority of these patients these reactions are negative. In every case desensitizing injections with the protein changed a positive fixation and precipitin reaction to a negative one. The peptone from horse dandruff and cat hair gave positive fixation in only two cases, and wheat leucosin failed to give fixation in any case, but the alkali meta-protein and the coagulated protein gave parallel fixation, and with the exception of leucosin the other wheat proteins gave parallel fixation. With S. pyogenes aureus antigen, cutaneous, complement fixation, and precipitin reactions are more comparable than with the proteins from horse dandruff, cat hair, and wheat.

CONCLUSIONS.

In this study of bronchial asthma, complement fixation and precipitin reactions, using the protein to which the patient is sensitive as an antigen, have been of no value in diagnosis, prognosis, or treatment. The cutaneous reaction, however, has proved to be of great value in prognosis and treatment and it seems the only practical and safe method at the present time for the diagnosis of the cause of bronchial asthma.

It is impossible to correlate the results from the cutaneous, complement fixation, and precipitin reactions with the proteins from horse dandruff, cat hair, and wheat; the results from these reactions are more comparable in the case of the bacterial proteins.

The cutaneous reaction is a demonstration of cellular sensitiveness; if we can accept the complement fixation reaction as an index of the presence or absence of serum antibodies, then many patients who are sensitive to a protein have no

antibodies for this protein in their serum and in the serum of patients who do seem to have antibodies they are present in small numbers. Furthermore, treatment in these cases with desensitizing injections of the antigen is followed by a decrease or absence of the serum antibodies.

The wheat albumin, leucosin, gave no positive fixation in any case, and the protein, peptone from horse dandruff, and from cat hair gave positive fixation in only one instance. The other proteins in horse dandruff gave parallel fixation and the same was true of the other proteins in wheat.

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PROGRESSIVE MUSCULAR OSSIFICATION (PROGRESSIVE OSSIFYING MYOSITIS) — A PROGRESSIVE ANOMALY OF OSTEOGENESIS.*

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Records of a small number of instances of progressive ossification of the muscular system have accumulated during the last century and a half and several medical museums contain skeletons in which massive bands of bone occupying the site of muscles are attached to the vertebræ, ribs or pelvis and often rigidly unite them to the bones of the arm or thigh. In 1741 John Freke described in the Philosophical Transactions the occurrence of a thick, bony mass which had grown upon the back of a man during three years and extended from the cervical vertebræ to the sacrum and laterally to all parts of the back so that it formed a kind of bony carapace. Other instances of ossification usually beginning during the first years of life and slowly implicating one muscle or group of muscles after another have been described. The disease derives peculiar interest from the circumstance that a large proportion of those affected exhibit an anomaly of the great toes and in some instances of the thumbs. Both great toes are of small size, not extending beyond the first inter-phalangeal joint of the second toe and often only one phalanx is recognizable.

The disease was first accurately described by Münchmeyer,[1] who in 1869 collected from his own experience and that of others twelve cases. He used the same *myositis ossificans progressiva* proposed by Von Dusch, since he regarded the condition as a constitutional disease associated with inflammation of muscles and characterized by slow progress with periodic exacerbations. There is swelling and perhaps pain at the site of the affected muscles and the overlying skin

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may become edematous. The local attack is occasionally accompanied by a mild febrile reaction which may persist for some time. The swelling disappears, the muscle is firm and may become rigidly ossified from origin to insertion. The muscles of the back and neck are usually first affected; the muscles of the upper extremities and of the thigh, the lumbar muscles, and the muscles of mastication may be partially or completely replaced by bone. Other muscles may be affected. There may be complete fixation of the head or jaw; the shoulder or hip joints may become rigid. Münchmeyer describes three stages of the muscular lesion: (a) inflammatory swelling, (b) fibrous induration with excessive growth of intermuscular and tendinous connective tissue, and (c) ossification.

The histological changes which occur in the muscles are described by Cahen.[2] He has found that the muscle fibers lose their cross-striations, disappear, and are replaced by fibrous tissue in which bone is laid down. Cartilage may be present and endochondral bone formation occurs. Persisting muscle cells may be found in narrow spaces between lamellæ of bone. Goto[3] has removed parts of muscle at various stages after the onset of local change and has reached the conclusion that the primary seat of the disease is the fibrous tissue of the aponeuroses, tendons, periosteum, and at times the ligaments, particularly of the vertebral column. The interstitial tissue of the muscle which other observers have believed to be first affected is ossified by extension from the parts which have been named. Goto suggests the name *hyperplasia fascialis ossificans progressiva*.

Rarefaction of the bones of the skeleton in association with the disease was noted by Krause and Trappe[4] in X-ray plates. They suggest that excessive bone formation in connective tissue occurs at the expense of the skeletal bones. Krause and Trappe in 1907 have collected sixty cases from the literature of the subject. Of those in which there was a record of the age of onset, ossification of muscles began thirty-eight times in the first decade, thirteen times in the second decade, and only once after the age of twenty years.

Boys are more frequently affected than girls in the proportion of three to two. The patient may live ten, twenty or more years, but death usually occurs at an early age, for the oldest of thirty-eight individuals with the disease of whom records have been collected by Pincus[5] was thirty years. Goto in 1913 collected six cases not included among those of Krause and Trappe.

A large proportion of those affected with this progressive ossification of muscles exhibit a congenital anomaly of the great toes, which is rarely if ever found in the absence of the disease. The occurrence of this anomaly was first recognized by Helferich[6] in 1879. The great toe was much shorter than the others (*microdaktylie*) and one phalanx was wanting. Jüngling[7] has analyzed the occurrence of this and related anomalies in forty-three cases of progressive ossifying myositis. In thirty instances, namely, approximately seventy per cent of those affected with the disease, there were anomalies of the toes or fingers distributed as follows: of the great toes alone, thirteen instances; of the great toes and of the thumbs, eleven instances; of the great toes, of one thumb, and of another finger, two instances; of fingers alone, one instance. Diminution in the size of the great toe occurred in association with several types of malformation. The great toe was in some instances diminished in size although it consisted of two distinct phalanges, but much more frequently there was ankylosis of the interphalangeal or metatarso-phalangeal joint or complete absence of one phalanx. Diminution in the size of the thumb was associated with changes analogous to those in the great toe. Jüngling cites three instances in which there were anomalies in some other part of the body, namely: (1) dwarfing of the penis, (2) absence of both lobes of the ears and of two upper incisors, or (3) hypospadias.

The skeleton of an old man suffering with progressive ossifying myositis is described because it exhibits the remarkable association of a congenital anomaly of the great toe with abnormal ossification of muscles, aponeuroses, and ligaments.

With relatively little muscular ossification the patient survived until the age of seventy, whereas most of those suffering with the disease have died in childhood or in early adult life.

The skeleton of a man aged seventy years was obtained through the kindness of Prof. Roy L. Moodie of Chicago. Little is known concerning the history of the individual who for a long time before his death was an inmate of an almshouse.

Skull. — The calvarium is thin and light in weight and the diploe is loose in texture. Upon the inner surface are deep pockets for the Pacchionian corpuscles and numerous smaller openings communicating with the spaces of the diploe. In many places the frontal and parietal bones are translucent and the orbital plates are formed by a thin shell of bone which is almost transparent. There is a well-defined frontal suture extending from the coronal suture to the inferior margin of the frontal bone. The coronal and adjacent part of the lambdoid sutures are partially obliterated.

There is no ankylosis at the temporal-maxillary joint, but the lower jaw is rigidly fixed by two bony masses occupying the position of the left pterygoid muscles (Plate XIX., Fig. 1). The upper mass consists of dense bone resembling in texture that of the inferior maxilla; it measures 1.3 centimeters from above down and approximately .6 centimeter in thickness. It arises, like the internal pterygoid, from the lateral pterygoid plate which is merged into it, from the pyramidal process of the palate, and from the adjacent part of the tuberosity of the superior maxilla. The plate of bone extends downward and backward to the anterior margin of the neck of the condyle of the inferior maxilla at the site of attachment of the internal pterygoid, but its union to the inferior maxilla is not limited to the site of attachment of the pterygoid muscles, for it is attached to the ramus of the inferior maxilla just behind the anterior border and in front of the lingula. A second rounded rod of bone .6 centimeter in diameter is fused with the first at its attachment to the lower end of the lateral pterygoid process, to the pyramidal process of the palatine, and to the tuberosity of the superior maxilla. It extends obliquely downward, outward, and backward, and is fused by a flat fan-shaped end with the inner surface of the ramus of the inferior maxilla just in front of the angle of the jaw. The position and attachments of this rod of bone correspond with those of the internal pterygoid muscle.

A groove which crosses the inner aspect or the mass representing the external pterygoid was doubtless occupied by the internal maxillary artery which in this instance appears to have been situated median to the external pterygoid and not in the more usual position external to the muscle. Foramina for branches of the artery penetrate the newly formed bone.

Vertebræ. — The anterior edges of the articular surfaces of the cervical vertebræ project as irregular lips of bone. From the adjacent articular

surfaces of the sixth and seventh vertebræ to the right of the median line there are projecting ridges of bone extending forward about .7 centimeter. The head of the seventh rib is ankylosed by bony union with the body of the corresponding thoracic vertebra and its neck is fused with the transverse process. The head and neck of the eighth rib is similarly ankylosed with the body and transverse process of the eighth thoracic vertebra (Plate XIX., Fig. 2). A rounded mass of bone continuous with the rib covers a considerable part of the lateral surface of the body of the vertebra. Extending downward from this mass are two thick scales of bone attached to the lateral surface of the body and extending over the intervertebral joint in contact with, but not attached to the body of the eighth vertebra.

The anterior and lateral edges of the articular surfaces of the bodies of the lumbar vertebræ form projecting lips from which flat osteophytes project often one-half to one centimeter. From the lower lip of the fourth lumbar vertebra and the upper lip of the fifth are bony projections which are in contact.

Ribs and Sternum. — Connecting the fifth and sixth ribs on the right side, 2.5 centimeters from their heads, and connecting the sixth and seventh ribs somewhat nearer the vertebral column are broad bands of compact bone about 2 centimeters across (Fig. 2). Parallel with the ribs and upon the anterior surface of the new bone midway between the two ribs is a ridge with a shallow groove on either side. On the left side, uniting the fourth and fifth ribs about 2.5 centimeters from their heads, is a band of bone which has similar markings upon its anterior surface.

The articular surfaces of the sternum have irregular projecting edges. The partially ossified cartilage of the first ribs is firmly attached to the bone. Ossification is progressing from the ribs into the cartilages attached to them.

The lips of the articular surfaces at the sternal ends of the clavicles are prominent and irregular. The coracoid tubercles are very conspicuous and the attachment of the deltoid is marked by irregular bony projections.

Bones of the Shoulders and Upper Extremities. — Of the shoulders and upper extremities there have been preserved the left scapula and humerus, the right ulna, and the bones of the hands. Ridges marking the attachment of muscles and of ligaments are prominent and there are irregularly projecting lips at the margins of articular surfaces; the bones although light in texture are much less porous than those of the lower extremity. The acromion process is ununited with the spine of the scapula. No deformity of the bones of the hand is found.

Pelvis. — The bone is porous and light in texture. The attachments of muscles, fascia, and ligaments are marked by projecting lines and tubercles. The external margin of the crest of the ilium affording attachment to the fascia lata is especially prominent. There are conspicuous projecting tubercles at the sites of attachment of the psoas parvus and of the adductor longus muscles. On the left side there is bony ankylosis at the upper part of the sacro-iliac synchondrosis. The edges of the acetabular fossæ form irregular projecting lips.

Femora. — There is advanced osteoporosis and an impacted fracture has occurred through the greater and lesser tuberosities. The head and neck of the bone are directed at a right angle to the shaft almost directly backward. The linea aspera is unusually prominent and projecting spicules of bone occur at the sites of attachment of the gastrocnemius muscles. The left femur has similar projections. An irregular ridge of bone 1 centimeter in height is found on the linea aspera just below the middle of the shaft.

Tibia and Fibula. — These bones are more irregular in outline than any of the long bones. Rough areas with spicules projecting upward in the direction of the muscle mark the attachment of the sartorius and semitendinosus muscles and of the patellar ligaments. The popliteal line is marked by spicules projecting .5 centimeter from the bone. Ossification of the interosseus membranes have occurred as irregular lamella and spicules extending from one bone toward the other. On the left side this new-formed bone forms several bridges firmly uniting tibia and fibula (Plate XX., Fig. 3). Ossifying periostitis over the shafts of both fibulæ has caused considerable thickening of these bones.

Bones of the Right Foot. — The tarsal bones are porous and friable. The surfaces are rough with low projections most conspicuous at the margins of articular surfaces and at the site of ligamentous attachments. The distal end of the first metatarsal bone is enlarged and very irregular in outline (Plate XX., Fig. 4). Projecting downward and backward from the head of the bone is an irregular osteophyte consisting of spongy bone 1 centimeter in length, and upon the under surface of the head are numerous projecting spicules of bone. Ankylosed with the upper and inner aspect of the articular surface is a small bony projection representing the two terminal phalanges of the great toe (Fig. 4). It is 3 centimeters in length, projecting upward in a position of hyperextension and is much smaller than the first phalanx of the second toe. Near the midpart of this bone is a foramen giving the phalanx an appearance as if bifurcated and again reunited. The bone ends in an ill-defined enlargement having the appearance of the end of a terminal phalanx. The bones representing the phalanges of the great toes do not extend beyond the first interphalangeal joint of the second toe.

Bones of the Left Foot. — The bones have the same porous texture exhibited by those of the right foot. The head of the first metatarsal bone is absent and the end of the bone is eroded. For a distance of 2 centimeters from the end of the bone the surface of the shaft is covered by a thin layer of porous bone. The appearance of the metatarsal bone suggests that the head with the great toe has been amputated and periostitis with superficial new formation of bone has occurred near the site of operation.

In a man aged seventy years the pterygoid muscles on the left side have undergone partial ossification, causing

ankylosis of the jaw; there is ossification of the interosseous membranes between tibiæ and fibulæ, ossification of ligaments, particularly of the vertebral column, and ossification at the site of attachment of muscles and fasciæ. The bones of the great toe on the right side are imperfectly developed, exhibiting an anomaly which has been found in seventy per cent of those suffering with progressive ossification of muscles. The great toe on the left side appears to have been amputated, presumably on account of a similar deformity. The bones exhibit other anomalies. Bridges of bone of similar form and even contour unite the fifth and sixth and the sixth and seventh ribs on the right side and the fourth and fifth ribs on the left side; there is a persistent frontal suture and the acromian process of the left scapula is not united with the spine. There is throughout the skeleton osteoporosis, which is most advanced in the lower extremities.

The greater number of those who have described instances of progressive ossification in muscles have designated the condition as myositis, believing that it is an inflammatory process. Münchmeyer states that the affected muscle becomes swollen and firm and the overlying skin exhibits well-defined edema, but is not red. There is mild fever. The microscopic changes described by Cahen, Goto, Stempel,[8] and others show that fibrous tissue proliferates and replaces muscle fibers; the cells of the connective tissue exhibit mitotic division and are numerous, but the lesion has not the character of inflammation. Cartilage has been present in the excised tissue and endo-chondral ossification occurs.

Virchow[9] regarded so-called progressive ossifying myositis as closely related to multiple exostosis and expresses the view that strands of bone grow from the skeleton into the muscles. Pincus[10] and Roth[11] have maintained that the periosteum has an important part in the new formation of bone. Mays,[12] who performed autopsies on two instances of the disease, states on the contrary, that bony masses may have their origin in tendons and fascia, and in connective tissue within or between muscles. Krause and Trappe

found numerous bony masses at a distance from the bones; the contours of the bones demonstrated by X-ray plates were entirely smooth. Stempel, who found evidence of nuclear division within cells of the connective tissue of muscle removed eight weeks after the onset of local changes, regards the lesion as a true tumor. The development of bone in masses corresponding to the form of muscles, ligaments, and tendinous attachments, and the well-defined limitation of growth exhibited by the newly-formed tissue do not suggest a resemblance to neoplasm.

The most remarkable character of this progressive ossification of muscles is its association with an unusual anomaly, namely, a retardation of the development of the bones and other parts of the great toes and occasionally of the thumbs. In the case which has been described other anomalies of the skeleton have been found. There is nothing to suggest that the anomalies associated with progressive ossification of muscles are referable to an inflammatory disease of fetal life and histological study of the changes found in the affected muscles does not indicate the presence of myositis. The normal course of osteogenesis has undergone a disturbance which has had its origin in embryonic life. This perversion of the cells concerned in osteogenesis persists and manifests itself by progressive new formation of bone throughout life in situations in which bone is not normally formed.

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EXPLANATION OF PLATES XIX. AND XX.

Plate XIX., Fig. 1. — Showing ossification of the pterygoid muscles on the left side. There is a persisting frontal suture.

Fig. 2. — Vertebral column and ribs showing anomalous bony union between fifth and sixth, and between sixth and seventh ribs on the right side, ankylosis of the head of the sixth rib, and scales of new-formed bone upon the bodies of vertebræ.

Plate XX., Fig. 3. — Showing ossification of the interosseous septum between the tibia and fibula on the left side.

Fig. 4. — Showing anomalous development and ankylosis of the phalanges of the great toe on the left side.

[I am indebted to Dr. W. S. Thomas for the accompanying photographs.]

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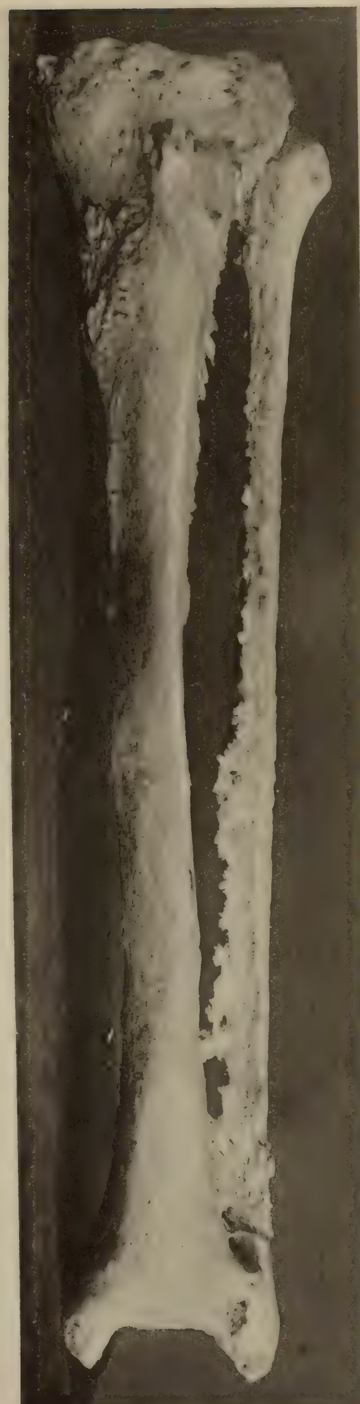

3

HYPERTROPHIC CHONDRODYSTROPHY IN INFANCY AND ADOLESCENCE — A PROGRESSIVE ANOMALY OF OSTEOGENESIS.*

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The disease now usually designated chondrodystrophy or achondroplasia is characterized by disturbance of the growth of cartilage associated with abnormal development of those bones which are formed within cartilage. The arms and legs remain short because replacement of cartilage by bone does not proceed with normal activity at the epiphyses and there is early cessation of endochondral ossification. The bridge of the nose is retracted because the base of the skull which is laid down in cartilage does not attain its usual length. It is now recognized that the disease which was formerly designated fetal rickets has no close similarity to rickets.

In most instances death occurs at or shortly after birth, but should the child survive continued life is possible. Apert and Lemaux[1] describe a case in which death occurred at the age of seventy years. Chondrodystrophic dwarfs are recognized by their short arms and legs in contrast to their normally-developed bodies; the apparently large forehead projects over the retracted root of the nose. Mental development is not retarded.

The histological changes in cartilage and bone with chondrodystrophy have been fully described in the publications of Kaufmann.[2] The zone of proliferating cartilage cells at the site of ossification is narrow and the arrangement of the cells in rows is ill defined. In the cartilage there is a narrow zone of calcification which is broken by spaces containing bone marrow. The columns of newly formed bone are irregularly disposed and tend to develop in breadth rather than in length.

In a monograph on Chondrodystrophia fetalis (1892)

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Kaufmann has described thirteen fetuses affected with the disease. In one group the cartilage is torpid and fails to exhibit normal growth; the growth of the endochondral bones is retarded. This condition is designated Chondrodystrophia hypoplastica. In a second group retardation of physiological development is associated with softening of cartilage. This condition previously described by H. Müller[3] and by Kirchberg and Marchand[4] (Mikromelia chondromalacia) is named by Kaufmann Chondrodystrophia malacica. In one fetus described by Kaufmann the bones were shorter than normal, the diaphyses were straight, but the cartilaginous epiphyseal ends showed colossal thickening, giving them often a mushroom shape. Microscopic examination demonstrated the absence of the usual arrangement of cartilage cells in orderly columns at the epiphyseal line. The irregularly arranged cells, in places of large size, were separated by intercellular substance which was conspicuously striated. Somewhat irregularly disposed marrow spaces penetrated into the cartilage which in their neighborhood had undergone calcification. There were numerous giant cells. Bone formation was in progress upon the surface of the cartilage, but the presence of numerous giant cells often situated within indentations in the lamellæ of bone or in the calcified cartilage indicated that bone absorption occurred in association with this retarded bone formation. The porous character of the bones merited the designation osteoporosis interna. The skeleton is laid down, Kaufmann believes, in normal fashion and the part which is formed in cartilage growing reaches a certain size; then a disturbance of osseous growth occurs referable to changes (dystrophy) affecting the epiphyseal cartilage. It loses its physiological tendency to growth in the long direction of the bone, but assumes an undisciplined tendency to growth in all directions. Marrow spaces penetrate into the cartilage and endochondral bone formation does not cease entirely. Periosteal bone formation is not affected.

Instances of hypertrophic chondrodystrophia in the fetus have been described by Johannsen[5] and by Simmonds.[6]

Joachimsthal[7] has described a typical chondrodystrophic dwarf eleven years of age, who he believed was affected with the hypertrophic type of the disease. The height was eighty-three centimeters, equal to that of a child two and a half years of age. The arms and legs were very short, but there was no retraction of the nose. The epiphyses were greatly enlarged as in advanced rickets and X-ray examination demonstrated a wide, clear zone at the site of the epiphyseal cartilages, interpreted as excessive growth of cartilage. There was enlargement at the costo-chondral junctions and bowing of the tibiæ caused symmetrical genu varum. Fragenheim[8] doubts if this case should be regarded as an instance of hyperplastic chondrodystrophy and thinks that X-ray examination does not exclude the possibility that the patient suffered with associated rickets and chondrodystrophy of the usual atrophic type.

The following case is a typical instance of hyperplastic chondrodystrophy in a child who has lived to the age of three months and is apparently still in good health:

L. D., male aged 3 months, was brought to the St. Louis Children's Hospital on account of deformity of the joints. Through the kindness of Dr. Borden S. Veeder we have had opportunity to study the patient.

The father is in good health. The mother is well; her first child is a girl seventeen years old, who walked at the age of nine months and is perfectly developed; her second child is a girl nine years old, who was prematurely born at the end of the seventh month of pregnancy; she walked when six months of age. During this pregnancy the mother suffered with rectal hemorrhage after the third month of gestation. She was able to nurse the child only two months. The third child is a boy aged five years, who walked at the age of eleven months. A sister of the patient's mother has five children, of whom the youngest is said to have had "weak bones" when a baby. There is no other history of disease of the skeletal system in the family.

During the third month of pregnancy the mother complained of soreness on the right side of the abdomen and in the small of the back and of cramping pains in the right leg. At this time there was a discharge of blood from the rectum occurring at times as black clots, at times as a constant oozing of black blood. This continued during the remainder of pregnancy. There was very marked edema of the right leg and swelling of superficial veins. During the fifth month of pregnancy the mother fell

backward on her right side and subsequently soreness and bleeding were more severe. She states that she fell upon the abdomen during the seventh month and subsequently until the end of pregnancy pain and soreness on the right side of the body made walking very difficult. The urine was not tested for albumin. Labor with normal vertex presentation was not difficult. The baby was strong and weighed thirteen pounds. On the third day following labor the mother had convulsions during four hours and was taken to a hospital, where she was unconscious for two days and delirious during three weeks. The convulsions were said to be due to "kidney poisoning." Enlargement of the bones of the child was noted at birth, and the mother says that he is now beginning to use his arms and legs. He has been fed on condensed milk and has had a cough which began three weeks ago.

The child (Plate XXI., Fig. 1) is moderately well nourished and the musculature appears to be normal. There is a frequently-repeated hoarse cough. The temperature is normal. The ends of all the long bones are enlarged and the limbs are short. The body is apparently of normal length, but the thorax is narrow and the sternum is prominent and the costal angle is acute. There is slight enlargement of the costo-chondral junctions. Harrison's groove is present. There is marked scoliosis to the left in the lower thoracic and upper lumbar region together with quite definite kyphosis. The coccyx is very prominent, protruding 1 centimeter below the tuber ischii. Folds of skin suggest a rudimentary tail.

There is great enlargement of the upper and lower ends of the humerus on both sides. There is no limitation of motion of the shoulder joint. The extremities of the bones of the forearm are similarly enlarged (Fig. 1), the enlargement of the lower end of the ulna being especially conspicuous. The motion of the elbows and wrist is limited and there is a creaking noise when the joint is moved. The dorsum of the hand is longer than usual. Motion at the second inter-phalangeal articulations of all the fingers is limited. There is great enlargement of the head, neck, and trochanter of both femurs and of both condyles. The shaft of the bone is somewhat shortened. The condyles and tuberosities of the tibiæ and the heads of the fibulæ are greatly enlarged. The lower extremities of both bones are large and prominent. The motion of the hip joints is limited in abduction. Extension of the knee is limited and the ankle joints are rigid. Creaking sounds are heard on motion of the joints of the lower extremity, but there is apparently no associated pain.

The fontanelle is open and tension over it appears to be normal. Coarse mucous râles are heard over the chest. Red blood corpuscles number 5,400,000; white blood corpuscles, 17,200; polynuclear leucocytes are 64 per cent. Weight of body is 4,200 grams. January 10, 1917 — Note by Dr. Veeder. — The chest has an abnormal contour, being rounded probably as the result of scoliosis, abnormality of the bones of the chest, and marked kyphosis of the dorsal vertebræ. The interscapular space is narrow. Percussion over the lungs is resonant. A few râles are

heard over the posterior surface of the apices. The abdomen is prominent and enlarged. The liver extends slightly below the costal margin, but is not enlarged. The spleen is just palpable.

X-ray plates demonstrate the enormous enlargement of the ends of the bones of the arms (Plate XXII., Fig. 3) and of the legs (Plate XXI., Fig. 2). The immense cartilaginous masses which form the epiphyses of the long bones cast no shadows, but theends of the diaphysis of the humerus, radius, ulna, femur, tibia, and fibula are flared out to correspond with the hypertrophied cartilages. This is particularly noteworthy in the femora of which the lower ends, of mushroom-like form, have a diameter approximately equal to two-thirds of the length of the diaphysis. The upper ends of the diaphyses of the tibiæ are similarly enlarged and projecting. The wide spaces between the diaphyses of the femora above and the diaphyses of the bones of the leg below represent the greatly enlarged epiphyseal cartilages which form the knee. The sternal ends of the bony ribs are enlarged.

The measurements of the patient are as follows:	Centimeters.
Length	55
Top of head to umbilicus	34
Umbilicus to sole of foot	24
Top of head to perineum	39
Right acromion process to tip of fingers	18
Left " " " " " "	19.5
Right " " " olecranon process	10.5
Left " " " " "	11
Right olecranon " " wrist	8
Left " " " "	9
Right wrist to tip of fingers	8
Left " " " " "	8
Length of right 2d finger	4
" " left " "	4
Right great trochanter of femur to external condyle	10
Left " " " " " " "	9
Right external condyle " " " " malleolus	8
Left " " " " " " "	9
Right foot	9.3
Left "	10.5
Occipito-frontal circumference of head	39
Circumference of chest at nipple line	34.5
" " " " costal margin	35.3
" " abdomen at umbilicus	36.5
" " " " anterior superior spine	30
" " right knees	14.5
" " left "	15

Enlargement of the epiphyseal cartilages with the corresponding enlargement of the ends of the diaphyses of the

bones has produced changes in the long bones, which are identical with those found by Kaufmann in a premature fetus and designated by him hypertrophic chondrodystrophy. Comparison of the X-ray plates (Figs. 2 and 3) made from the patient with the plates of Kaufmann depicting macerated fetal bones demonstrates that the two conditions are the same. The lesion present at birth has occurred in a child which has survived three months and is increasing in weight. There is no definite evidence that the lesion has occurred in any other member of the family.

The case which will be described does not present some of the characters usually associated with chondrodystrophy. No retardation of the growth of the long bones has occurred. Nevertheless, there has been over-excessive growth of cartilage microscopically similar to that which is found with chondrodystrophy.

C. L., white male, aged 17 years, entered the Barnes Hospital complaining of difficulty in walking. His father died with injuries sustained in an accident at the age of seventy years, his mother fifty-five years of age, one brother, and one sister are living and well.

The patient has never been in good health. He says that he had "spasms" at the age of three months, but does not know the character of the illness. Enlargement of the wrist, ankles, elbows, knees, and joints of the fingers and toes followed. Until the last year and a half these joints have never pained or given other inconvenience, although he has been slightly lame since infancy as the result of some stiffness of both hips.

About four years ago his right leg was caught in a wagon wheel and badly twisted, but he does not think that it was broken. He was not confined to bed on account of the accident, but since this time the right leg and foot have been rotated slightly outward. He thinks that he had rheumatism about three years ago when both knees became swollen, red, and painful. He was in bed for three months and believes that his knees became somewhat stiff and somewhat larger than before. One and a half years ago, following exposure incident to swimming early in the spring, there was much pain in the right hip and after five or six months both hips became so stiff that, as he believes, there was no motion in either hip. He can walk only with aid of a cane.

The patient has frequently suffered with headache and dizziness. At the age of fourteen he left school having attained only to the fourth grade (usually passed at twelve years).

The patient is an adolescent male of small stature, and somewhat poorly nourished. He is not very intelligent, becomes confused and frequently makes contradictory statements. The muscles are small and undeveloped. All of the visible joints are enlarged (see Plate XXII., Fig. 4). The head of the humerus on each side is enlarged, but active and passive movement is not restricted. The elbows are greatly enlarged and flexion of the right is limited to a right angle. Slightly greater flexion of the left is possible. Rotation of the right forearm is not possible. There is slightly limited rotation of the left forearm. The wrists are much thickened and permit only limited flexion and extension. The inter-phalangeal joints of all the fingers are enlarged and fusiform in shape and complete closure of the hands is not possible.

The pelvis is tilted so that the right crest of the ilium is higher than the left. The right hip is completely ankylosed and in a position of partial flexion, slight abduction, and external rotation. The joint is not acutely tender although there is slight pain on deep pressure. The left hip permits slight motion in all directions and is abducted, due to tilting of the pelvis. The knees are greatly enlarged; the right permits little motion, the left is freely movable. The ankles are much enlarged and movement is restricted. The inter-phalangeal joints of both feet are slightly enlarged. The patellar reflex is more active than usual.

Examination of the chest and abdomen discloses no abnormalities. There is no sugar, albumin, nor casts in the urine. The blood contains 5,420,000 red blood cells, 9,050 white blood cells to the cubic millimeter, and 95 per cent of hemoglobin.

Röntgen-ray examination of the elbow shows immense enlargement of the ends of the bones composing it. The ends of the metacarpal bones and of the phalanges (Plate XXIII., Fig. 6) are considerably enlarged, and the epiphyses, save those of the thumb and distal phalanges, are not united to the shafts. The head of the radius (Plate XXIII., Fig. 7) is a very broad, flat disc, situated upon the enlarged end of the shaft. The olecranon is of very great size. The enlarged epiphyses at the lower end of the radius and ulna are not united to the adjacent enlarged ends of the diaphyses. The head and neck of the femur and the great trochanter (Plate XXIII., Fig. 8) are of immense size. The texture of the bone is very loose.

Examination of the knee joint shows great enlargement of the ends of the femur, tibia, and fibula, this enlargement involving the epiphyses and the adjacent parts of the diaphyses. The epiphyses are not united to the shaft. The epiphysis at the lower end of the tibia (Plate XXIII., Fig. 9) is not united to the shaft. The epiphysis and the adjacent end of the diaphysis is greatly enlarged.

Following are measurements of the patient compared with normal measurements for the same age taken from the anthropometric table of J. W. Seaver:

	Patient.	Average for Age of Patient.
Height	1,363	1,645
Height to notch of sternum	1,030	1,345
Height to navel	970	974
Height sitting	730	868
Height to knee	405	422
Length of head and neck	333	300
Length of trunk	397	568
Length from shoulder to elbow	380	346
Length from elbow to tip of fingers	453	437
Length of thigh	561	655
Length of right foot	257	240
Breadth of head	115	149
Breadth of neck	111	98
Breadth of shoulders (at level of achromion)	397	382
Breadth of chest	251	247
Breadth of waist	240	234
Breadth of hips	340	302
Girth of head	563	550
Girth of neck	345	329
Girth of chest	835	800
Girth of waist	680	677
Girth of hips	865	841
Girth about right biceps	235	262
Girth of right elbow above condyles	213	208
Girth of right elbow over condyles	283	224
Girth of right forearm	220	227
Girth of right wrist below styloid process	180	155
Girth about left biceps	250	
Girth of left elbow above condyles	213	204
Girth of left elbow over condyles	275	220
Girth of left forearm	233	235
Girth of left wrist below styloid process	185	154
Girth of right thigh	430	474
Girth of right knee	370	333
Girth of right calf	275	323
Girth of right ankle above malleolus	210	
Girth of right ankle over malleolus	270	
Girth of right instep	255	218
Girth of left thigh	385	466
Girth of left knee	383	334
Girth of left calf	295	323
Girth of left ankle above malleolus	235	
Girth of left ankle over malleolus	275	
Girth of left instep	255	218

The height of the patient is less than the average height for the same age, but the length of the trunk is relatively less than the total height.

The length of the bones of the leg in correspondence with the small stature is less than usual, but the bones of the arms on the contrary are longer than those of the average individual whose height is considerably greater than that of the patient. The girth about the muscular parts of the arms and legs is uniformly less than normal, but the girth of the limbs in the neighborhood of the joints is uniformly much increased.

On December 2 an operation was performed (Allison) with the purpose of overcoming the ankylosis of the left hip joint. An incision was made over the great trochanter. The muscles attached to the trochanter were turned upward and the capsule was incised. The head of the femur, which was very large, was thus exposed in the wound of operation. The acetabulum was found to be very deep and the cartilage covering it was in general smooth, but there was some erosion of cartilage in the neighborhood of the ligamentum teres. The articular cartilage of the head of the femur and some of the adjacent bone was removed and the end, rounded off, was dropped back into the acetabulum. The great trochanter was held in place with heavy silk ligatures. The fascia and skin were closed with silk sutures and the patient was put in a spica cast with the hip approximately straight. Recovery from the operation was rapid.

On January 18 the position of the right hip was as follows: there is flexion to about fifteen degrees and the thigh is held in external rotation in a straight line with the trunk; motion in adduction to ten degrees, in abduction to twenty degrees, and in flexion to twenty degrees is possible. The right lower extremity is about one inch longer than the left. The patient gets about freely with crutches and has been discharged from the hospital.

The tissue removed at operation in several pieces consists of the articular cartilage of the head of the femur and the underlying bone. When these pieces are placed together the head (Plate XXII., Fig. 5) is found to be approximately 8.5 centimeters in diameter. The articular cartilage is about two millimeters in thickness and shows no erosion, but in places there are low rounded elevations where the cartilage is four or five millimeters in thickness. The underlying bone is very soft and loose in texture and can be readily cut with a knife. At one point within the bone are small masses of cartilage apparently having no connection with the articular cartilage on the surface and extending as much as 1.3 centimeters below it. Approximately two centimeters below the articular cartilage is a line of cartilage separating the head from the neck of the bone. The marrow below this

epiphyseal cartilage is darker in color than that above it and contains less fat.

The articular cartilage covering the head of the femur in a narrow superficial zone contains cartilage cells which are spindle-shaped and have an oval vesicular nucleus usually containing one, or occasionally two, nucleoli. The matrix is here striated, the striations being parallel to the surface. Below this zone is a much wider one of less compact cartilage with conspicuously striated groundwork. The cells are shrivelled in appearance, being irregularly spindle-shaped or stellate with deeply-stained homogeneous nuclei which have an irregular contour and appear to be shrunken. Many of these cells contain large or small vacuoles. They are usually collected into groups and between individual cells and about the groups the groundwork of the cartilage consists of a palely-stained network of fibrillæ. Fuchsin stains very deeply the more compact fibrillated groundwork between the groups of cells. Fibers passing through clear spaces in the deeper part of this zone are disposed at right angles to the surface of the cartilage. In the lowermost part of the articular cartilage (Plate XXIV., Fig. 10) the cells are arranged in elongated groups which are clearly defined in sections stained with hematoxylin by the deep blue stain of the surrounding groundwork (calcium deposit in capsule of cells). These cells are oval with abundant cytoplasm and a fairly large round or oval nucleus. The cells in such groups are frequently arranged in rows of from three to six members. Specimens stained with fuchsin show that the fibrils within the ground substance in this region are perpendicular to the articular surface and therefore parallel with the rows of cartilage cells.

Very short marrow cavities containing cellular connective tissue and blood vessels penetrate from the under surface into the articular cartilage. Along this surface there is an almost continuous thin layer of bone which surrounds the ingrowing marrow cavities. The bone is covered by a layer of cubical or occasionally flat osteoblasts. In a few places the irregular layer of bone and the corresponding line of

osteoblasts is interrupted and in these places multinucleated giant cells are in contact with the cartilage, often lying in lacunæ hollowed out in its substance. Bony trabeculæ forming the head of the femur are continuous with the layer of bone in contact with the articular cartilage. These trabeculæ are slender and sparsely scattered and the space between is filled with vascularized adipose tissue containing very few cells.

Epiphyseal cartilage (Plate XXIV., Fig. 11) separating the head of the femur from the neck is from .5 to .7 millimeter in thickness and is more deeply stained with hematoxylin (calcium) than the articular cartilage. Covering that surface of the cartilage which is directed toward the epiphysis is an irregular layer of compact bone in places nearly a millimeter in thickness. On the surface of this bone there are no osteoblasts or giant cells and marrow cavities do not penetrate from it into the cartilage. A zone of cartilage in immediate contact with this bone stains in places deeply with hematoxylin (calcium); its cells are swollen and about them the matrix is more deeply stained than elsewhere. Below this zone the matrix is homogeneous and contains few cells. On the side of the epiphyseal cartilage directed toward the neck of the femur, bone formation and absorption are active and both cartilage and bone resemble the same tissue upon the under surface of the articular cartilage. The cartilage cells are usually angular in outline and have two or three pointed processes; their nuclei stain homogeneously and deeply and appear to be shrivelled. They are scattered in a conspicuously striated matrix consisting of a loose network of fibrils. In a specimen stained with acid fuchsin (Van Gieson's stain) the matrix surrounding these shrivelled cells is almost wholly unstained, whereas the matrix elsewhere is stained deep red. In contact with the bone of the neck the cartilage contains a few plump normal cells, but there is no arrangement of cells in rows. Patches of bone form a nearly continuous thin layer upon the surface of the cartilage. A layer of osteoblasts covers this bone in part, whereas elsewhere osteoblasts are absent and large multinucleated giant

cells often occur in lacunæ upon the surface of the bone. In places the layer of bone is broken and osteoblasts or giant cells are in immediate contact with cartilage. Numerous marrow spaces penetrate a short distance into the cartilage and about them bone formation or absorption is in progress. Bone of the neck of the femur differs in texture from that of the epiphysis; bony trabeculæ are stouter and closer together. In the marrow cells are much more numerous and form about one-third of the tissue, the remainder being fat.

Cartilaginous masses embedded within bone are found below the articular cartilage (Plate XXIV., Fig. 12) and replace marrow between the trabeculæ. These masses of cartilage included within the bone are composed of striated matrix, varying much in the intensity with which it takes stains, and cells which are spindle-shaped, triangular or stellate with pointed processes, and deeply stained shrivelled nuclei. Vacuoles frequently occur within the cells. This cartilage has the loose texture seen in the mid-part of the articular cartilage (see above); in a rounded area about each cell the matrix remains nearly unstained either by hematoxylin (calcium) or by fuchsin (collagen fibrils), whereas between these areas it is somewhat more deeply stained, so that in many places the cartilage appears to be made up of globular masses of matrix each surrounding a cell. In several places scant bone formation occurs along the edge of an included cartilaginous mass, but elsewhere the cartilage is in immediate contact with bone marrow and fat surrounding it. The edge of the cartilage is lobulated and rounded projections of varying size push out into the surrounding tissue. There has evidently been an invasive outgrowth of cartilage, for on the one hand fat cells are included within the cartilaginous mass (Fig. 12), whereas on the other hand small isolated globules of cartilage are found at the edge of large masses and wholly isolated in the marrow tissue. In some instances these globules of cartilage represent a single cell (Plate XXIV., Fig. 13) surrounded by its zone of matrix. This ball of matrix is clear and almost unstained immediately about the cell, but

takes a somewhat deeper stain with hematoxylin or with acid fuchsin (Van Gieson's stain) about its periphery. The cartilage cell appears to have become isolated in the fat and here laid down matrix about itself.

Fibrous tissue forming a zone as much as two millimeters in thickness and separating the proliferating articular cartilage from the underlying bone is found in one section over an area about 1.5 centimeters in breadth. This tissue is in places richly vascularized by wide channels lined by endothelium and containing blood; these channels are arranged parallel to one another and at right angles to the articular cartilage. The fibrous tissue is dense and contains few cells. In several spots within this fibrous tissue and in the absence of cartilage there is bone formation. Surrounding what has the appearance of a marrow space there is a single or multiple layer of osteoblasts, outside of which is a circle of bone with calcified matrix and numerous bone corpuscles. This bone merges into osteoid tissue containing cells resembling bone corpuscles and devoid of calcium salts. Over this layer of fibrous tissue the articular cartilage is thrown up into a rounded projection .5 centimeter in thickness.

Abnormal formation of bone at the ends of the long bones involving the epiphyses and the adjacent part of the shaft has brought about great enlargement of the ends of these bones. This enlargement affecting the epiphyses was first observed at the age of three months at a time when the epiphyses were in large part cartilaginous. There has been no retardation of the growth of the long bones, but there is some retraction of the root of the nose. Röntgen-ray examination shows that the epiphyseal cartilages of the long bones with few exceptions (for example, those at the upper ends of the ulna and radius) are not united to the shaft. The longitudinal growth of the bones as indicated by measurements show considerable deviation from normal, the trunk being very short and the limbs relatively long. Microscopical examination of the articular and epiphyseal cartilages of the

femur demonstrate the occurrence of endochondral bone formation both upon the under surface of the articular cartilage and upon the diaphyseal side of the epiphyseal cartilage. Striation of the matrix of the cartilage and changes in the cartilage cells furnish further evidence that the cartilage has developed abnormally. The cartilage cells near the site of ossification have not assumed the normal arrangement in long rows perpendicular to the line of ossification, but occur in irregular groups or in short rows of from three to six members. There appears to be a tendency for the cartilage to proliferate laterally as well as longitudinally. The histological changes are similar to those described in association with chondrodystrophia of infants.

One case similar to that which we have described is recorded by Schorr.[9] A young mulatto girl aged sixteen years had suffered during five years with weakness, pains in the arms and legs, and difficulty in walking. The author briefly states that three sisters were affected with an analogous disease. The epiphyses of the long bones and the costo-chondral junctions were enlarged. The diaphyses of the long bones were not bent. The patient died with tuberculosis. The bones of the skull and the tibia were softer than usual and the bodies of the vertebræ and the ribs could be cut with a knife. There was a zigzag line of ossification between the rib and its cartilage. The cartilage between the upper epiphysis and the diaphysis of the tibia was irregular in outline and in places appeared as if it were made up of circumscribed pieces of cartilage. The intercellular substance of the cartilage was fibrillated. In places there was in progress the normal process of endochondral ossification, the cartilage cells being disposed in rows, but elsewhere there was no orderly arrangement. The lamellæ of bone were thinner than normal and formed a network with wide meshes (osteoporosis). In the spongy tissue at the end of the diaphysis of the tibia were found a number of islands of cartilage which had few cells and fibrillated intercellular substance. Schorr discusses the possibility that this case is an instance of late rickets, but finds that the histological changes at the

site of endochondral bone formation do not resemble those of rickets. He designates the condition chondrodystrophia adolescentium since it makes its appearance at puberty. It should not be identified, he thinks, with the form of chondrodystrophy which occurs in young individuals as the end result of a chondrodystrophic process having its origin in intrauterine life or in early infancy.

The great enlargement of the epiphyses of the long bones in the patient which we have described, having its origin in infancy, is similar to the enlargement occurring with the hypertrophic type of chondrodystrophia fetalis. Absence of dwarfing of the long bones does not exclude chondrodystrophy. Kaufmann has described two fetuses in which there was retraction of the root of the nose, but no diminution in the length of the bones of the arms and legs, although histological examination of the growing ends of the bones showed characters similar to those found in other instances of chondrodystrophia. In our patient the profile of the face with its projecting forehead and retracted nose suggest that there has been some retardation of the growth of the endochondral bones forming the base of the skull. The bones of the extremities on the contrary have attained unusual length.

The condition has some resemblance to the disease which has been described by Virchow in Die krankhaften Geschwulste and is usually designated multiple cartilaginous exostosis.[10] This disease is congenital and often hereditary, being transmitted by both the father and the mother. Ehrenfried [11] has recently employed the name hereditary deforming chondrodysplasia. It is characterized by the occurrence of multiple, often symmetrical cartilaginous exostoses upon those bones which develop in cartilage. In the long bones exostoses are more numerous at the site of endochondral bone formation between the epiphysis and diaphysis and often develop as outgrowths from the epiphyseal cartilage, but they are not limited to this situation.

Disturbance of growth of bones occurs and some of the affected individuals are greatly deformed dwarfs, the arms

and legs being shorter than usual and generally much altered in shape. There is bending of bones of the forearm due to retardation of the growth of the ulna out of proportion to that of the radius and extreme pes valgus may be caused by great retardation of the growth of the fibulæ. Endochondral bone formation occurs in a disorderly fashion; growth of cartilage is excessive and irregularly grouped cartilage cells give rise to abnormal outgrowths of bone. Isolated masses of cartilage may persist uncalcified within the head or shaft of the bone. Disorderly development of epiphyseal cartilage may be associated with retardation of the growth of long bones.

With hypertrophic chondrodystrophy there is, on the contrary, uniform enlargement of the ends of the long bones; growth of cartilage is excessive, but proceeds without the disorder which brings about the formation of exostoses. Nevertheless, there is inclusion of aberrant cartilage within bone. Embedded within the head of the femur beneath the articular cartilage we have found irregular masses of cartilage which exhibit only slight tendency to undergo calcification or ossification. This cartilage proliferates and cells penetrate between the surrounding cells of the marrow fat. In some instances balls of cartilage, each consisting of a sphere of matrix about the cell which formed it are found wholly isolated in the fat. With the growth of this cartilage fat cells may be included within it. Nevertheless, no excessive bone formation occurs and there is no tendency to form exostoses.

Chondrodystrophy of hypertrophic type characterized by excessive proliferation of cartilage may persist throughout adolescence and give rise to immense hypertrophy of the epiphyses and adjacent ends of the diaphyses. There is progressive proliferation of both articular and epiphyseal cartilage and bone formation occurs at the edge of the proliferating cartilages, but multiplication of cells fails to produce the regularly disposed rows of cells characteristic of the longitudinal growth of bone. Small areas of cartilage are found isolated within the bone which has been formed and by

proliferation this cartilage penetrates between the adjacent fat cell of the marrow. Absorption of bone accompanies its new formation and osteoporosis is a conspicuous feature of the disease. The bones may not be diminished in length and in one case which has been described the long bones are abnormally long in proportion to the trunk, but the projecting forehead suggests that the growth of the endochondral bone at the base of the skull has been somewhat retarded.

The foregoing observations establish the occurrence of hypertrophic chondrodystrophy as a disease of post-fetal life, and show that associated abnormal endochondral osteogenesis may persist throughout adolescence. The disease is an inborn and progressive anomaly of endochondral bone formation.

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EXPLANATION OF PLATES XXI. TO XXIV.

PLATE XXI., FIG. 1. — Infant aged three months with hypertrophic chondrodystrophy showing enlargement of the ends of the bones.

FIG. 2. — X-ray plate of the legs of an infant with hypertrophic chondrodystrophy showing great enlargement of the ends of the diaphyses of the long bones.

PLATE XXII., FIG. 3. — X-ray plate of the arms and thorax of an infant with hypertrophic chondrodystrophy showing enlargement of the diaphyses of the long bones of the arms and of the bony ends of the ribs.

FIG. 4. — A boy aged seventeen years with hypertrophic chondrodystrophy showing enlargement of the joints.

FIG. 5. — Showing the greatly enlarged head of the femur removed at operation. Compare with the head of a normal femur shown below.

PLATE XXIII., FIG. 6. — X-ray plate of the hand of a boy with hypertrophic chondrodystrophy showing enlargement of the ends of the radius and ulna, metaphalangeal and phalangeal bones.

FIG. 7. — X-ray plate of elbow of a boy with hypertrophic chondrodystrophy showing enlargement of the ends of the bones. The enlargement of the head of the radius is particularly well shown.

FIG. 8. — X-ray plate of the head and trochanters of the femur in a boy with hypertrophic chondrodystrophy showing great enlargement and rarefication of the bone.

FIG. 9. — X-ray plate of the ankle of a boy with hypertrophic chondrodystrophy showing enlargement of the ends of the tibia and fibula.

PLATE XXIV., FIG. 10. — Photomicrograph of the articular cartilage of the head of the femur showing line of ossification and distribution of cartilage cells in short, irregular rows just above this line.

FIG. 11. — Epiphyseal cartilage of the head of the femur showing line of ossification on the under surface with cartilage cells irregularly disposed just above this line.

FIG. 12. — Photomicrograph of the articular cartilage of the head of the femur with masses of isolated cartilage immediately below. The matrix of the articular cartilage is striated. The isolated masses of cartilage penetrate into the adipose tissue of the bone marrow, and in places fat cells are included within them.

FIG. 13. — Photomicrograph with high power of cartilage isolated within the bone. Single cartilage cells are isolated in fatty marrow of the bone.

[We are indebted to Dr. W. S. Thomas for the accompanying photomicrographs.]

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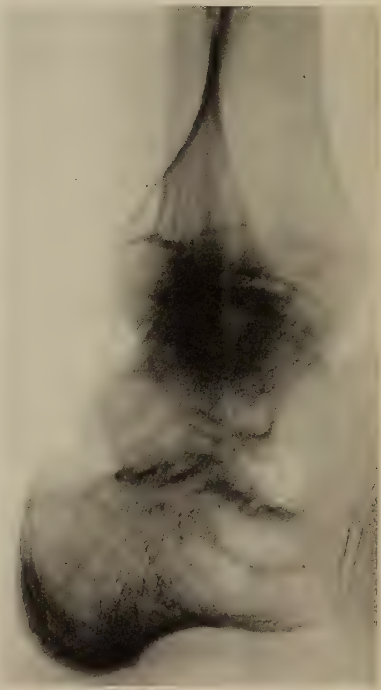

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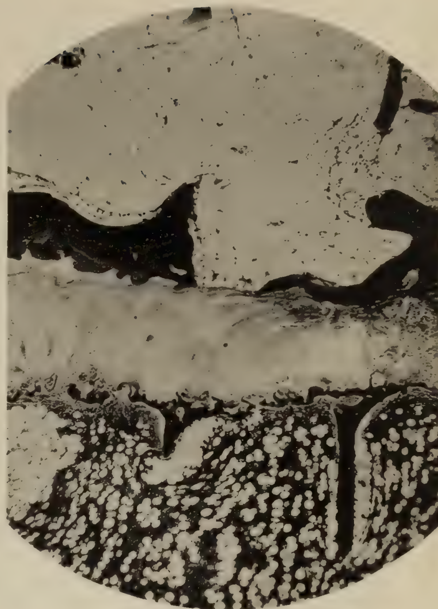

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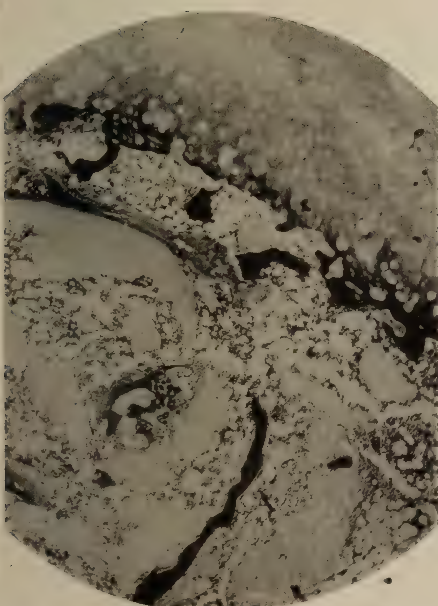

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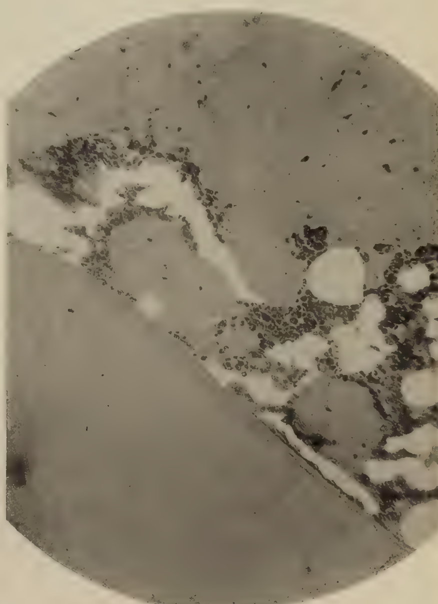

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STUDY XIII.

The relationship between the cutaneous reaction, serum agglutination tests and bacteriological examination of the sputum and nasal secretions in determining the part Staphylococcus pyogenes aureus and albus may play in the cause of bronchial asthma.*

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In this study of bronchial asthma, we are applying to each patient all the different laboratory tests which are at our command in order to determine a possible bacterial cause of the disease in each case. The cutaneous reaction, which was discussed in Studies III., IV., V., and XII., has proved to be of as great value in determining the cause of asthma from bacterial proteins as from other proteins. In addition to the cutaneous reaction, serum agglutination tests have been done and bacteriological examinations of the sputum and nasal secretions have been made to see if they would give additional information as to the bacteriological causes of asthma and would substantiate the results of the cutaneous reaction. The previous paper, Study XII., discussed the relationship between the cutaneous reaction and serum complement fixation and precipitin reactions. The present paper is a discussion of the serum agglutination tests with the effect on these of vaccine treatment and of the results of bacteriological examinations of the sputum and nasal secretions. In Study III. it was shown that S. pyogenes aureus and albus were the bacteria which usually gave positive cutaneous reactions, in fact other bacteria rarely gave one, and in Study I. it was shown that agglutination tests between serum and bacteria other than S. pyogenes aureus and albus

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were too unsatisfactory to be depended upon, so that in this paper we are concerned only with S. pyogenes aureus and albus.

Agglutination tests have been done between the serum of one hundred and twenty-five patients with bronchial asthma and several strains of S. pyogenes aureus and albus; the serum of seventy-five of these patients did not agglutinate S. pyogenes aureus at all, the serum of twenty patients agglutinated this organism in a dilution of 1–50 or less, and the serum of thirty patients agglutinated this organism in a titer of 1–100 or higher, and it is with these latter that we are concerned in this paper. The serum of none of these patients agglutinated S. pyogenes albus in a titer higher than 1–20, so that agglutination tests with this organism may be dismissed immediately. The technic of the agglutination tests and of the bacteriological examination of the sputum and nasal secretions has been described in Study I.

Protocol I.

Positive agglutination tests between S. pyogenes aureus and the serum of bronchial asthmatics who give positive cutaneous reactions with the protein of this organism and the effect of vaccine treatment with this organism on the agglutination test.

M. S., a woman aged 31, has had asthma for nineteen years. On April 11, 1916, cutaneous reactions were positive with the protein of S. pyogenes aureus, a strain of this organism was isolated from the sputum and the patient's serum agglutinated each of four stock cultures of S. pyogenes aureus in the following dilutions, — No. 1 = 1–200, No. 2 = 1–200, No. 4 = 1–200, and No. 5 = 1–200. Six treatments were given at weekly intervals with S. pyogenes aureus vaccine in amounts varying between 200 and 500 million at each time. In this work no autogenous vaccines were used. Sufficient quantities of vaccine were made at one time from the same stock culture of S. pyogenes aureus and albus to last throughout a year, so that the same vaccine was used with each patient throughout this paper. During this treatment the patient became free from asthma. Following four more similar treatments with vaccines, agglutination tests were repeated on June 29, 1916, with the same stock cultures of S. pyogenes aureus with the following results, — No. 1 = 1–50+, No. 2 = 1–20+, No. 4 = 1–50+, and No. 5 = O. After three more treatments with

vaccine varying between 200 and 400 million each time, on July 11, 1916, the cutaneous reaction with S. pyogenes aureus protein was slightly positive and the agglutination tests with stock cultures of this organism were as follows, — No. 1 = O, No. 2 = O, No. 4 = 1–50+, and No. 5 = O. After two more vaccine treatments, one of which was with 500 million and the other with 600 million, agglutination tests were repeated and were found to be negative with all four stock cultures. The patient had had no asthma for two months and the cutaneous reaction with S. pyogenes aureus protein was negative, therefore treatment was discontinued. On Nov. 10, 1916, the patient had an attack of asthma for ten days and at this time agglutination tests with the same four stock cultures of S. pyogenes aureus which were previously used were positive in the following dilutions, — No. 1 = 1–100, No. 2 = 1–150, No. 4 = 1–100, and No. 5 = 1–150. Following six treatments with S. pyogenes aureus vaccines in amounts varying between 250 and 400 million, the patient had no asthma. After five more treatments with vaccines during which time the patient had no asthma, agglutination tests were negative with the same four stock cultures of S. pyogenes aureus. In the previous paper (Study XII.) it was shown that complement fixation and precipitin reactions with S. pyogenes aureus antigen were negative in this case.

F. S. E., a man aged 35, has had asthma for two years. On May 18, 1916, the cutaneous reactions with the proteins of S. pyogenes aureus and albus were positive and agglutination tests with stock cultures of S. pyogenes albus were negative, but with S. pyogenes aureus they were positive in the following dilutions, — No. 1 = 1–150, No. 2 = 1–100, No. 4 = 1–100, No. 5 = 1–100, and No. 7 = 1–150. Neither S. pyogenes aureus or albus were isolated from the sputum. After four treatments at weekly intervals with S. pyogenes albus vaccine in amounts ranging between 200 and 700 million, the patient had no more asthma, the cutaneous reaction with S. pyogenes albus protein was much less positive, but the cutaneous reaction with S. pyogenes aureus protein was as positive as before. At this time, on June 20, 1916, agglutination tests were still negative with S. pyogenes albus and with S. pyogenes aureus they were positive in the following dilutions, — No. 1 = 1–50, No. 2 = 1–100, No. 4 = 1–50, No. 5 = O, and No. 7 = 1–50. Following three more treatments with S. pyogenes albus vaccine in amounts varying between 700 and 1,000 million, agglutination tests with both S. pyogenes aureus and albus were negative. Six months later (on Jan. 18, 1917) the patient had a return of asthma, cutaneous reactions with the proteins of S. pyogenes aureus and albus were positive, but agglutination tests with each organism were negative. Following two more treatments with S. pyogenes albus vaccine, the patient had no asthma.

M. M., a woman aged 44, has had asthma for fourteen years. On Sept. 13, 1916, cutaneous reactions were positive with the protein of S. pyogenes aureus and agglutination tests with four stock cultures of

this organism were positive in the following dilutions, — No. 1 = 1–200, No. 2 = 1–150, No. 4 = 1–200, and No. 5 = 1–200. During the next three months S. pyogenes aureus vaccines were given at weekly intervals in amounts varying from 200 to 550 million. During this time the asthma had greatly improved and only occasional slight wheezing was noted. At this time (Dec. 19, 1916) agglutination tests with the four stock cultures of S. pyogenes aureus which were previously agglutinated were negative.

P. McL., a man aged 53, has had asthma for three years. On Sept. 20, 1916, cutaneous reactions with S. pyogenes aureus protein were slightly positive, and agglutination tests with four stock cultures of this organism were positive in the following dilutions, — No. 1 = 1–150, No. 4 = 1–100, No. 8 = 1–200, and No. 13 = 1–150. During the next three months he was given at weekly intervals S. pyogenes aureus vaccine in amounts varying between 200 and 700 million. During this time the patient improved until he was free from asthma for a month, and at this time (Nov. 9, 1916) agglutination tests were positive in the following dilutions, — No. 1 = 1–50, No. 4 = 1–50, No. 8 = 1–100, and No. 13 = 1–20. At the end of the three months' treatment agglutination tests were negative with all cultures.

P. J. F., a man aged 48, has had asthma for twelve years. On Aug. 18, 1916, cutaneous reactions with S. pyogenes aureus were slightly positive, from the sputum was isolated S. pyogenes albus and agglutination tests with three stock cultures of S. pyogenes aureus were positive in the following dilutions, — No. 1 = 1–100, No. 8 = 1–100, and No. 9 = 1–50. Following treatment for a period of three months with S. pyogenes aureus vaccine, the patient had very little asthma and agglutination tests with the three stock cultures of S. pyogenes aureus were negative.

In the first case (M. S.), who had had bronchial asthma for nineteen years, S. pyogenes aureus would seem to be the cause, since during treatment with vaccines of this organism the patient became free from asthma and remained so for five months. When asthma returned the patient was again relieved following six treatments with S. pyogenes aureus vaccine and is still free two months later. During the first series of vaccine treatments, agglutination tests were done four times, so that the opportunity was presented for following the gradual change in these tests from positive to negative. Following ten vaccine treatments the agglutination tests changed from positive in a dilution of 1–200 to positive in a dilution of 1–50 or less. After three more vaccine treatments three of the four cultures became negative and

after an additional two more treatments agglutination tests were negative with all cultures. On the return of asthma, agglutination tests were again positive with S. pyogenes aureus in dilutions of 1–100 and 1–150, but following eleven vaccine treatments with this organism agglutination tests again became negative. It is interesting to note that each time agglutination tests became negative after practically the same number of treatments with approximately the same number of bacteria. Thus this patient gave positive cutaneous reactions with the protein of S. pyogenes aureus, the sputum contained this organism and the serum gave positive agglutination tests with it and negative complement fixation and precipitin reactions with an antigen made from it.

In the second case (F. S. E.), S. pyogenes albus would seem to be the cause of asthma, since during treatment with vaccines made from this organism, asthma was relieved and when, six months later, asthma returned it was relieved a second time by similar treatment. The patient gave positive cutaneous reactions with both S. pyogenes aureus and albus proteins, but the serum agglutinated only the former; neither organism was isolated from the sputum. It is interesting that following four treatments with S. pyogenes albus vaccine agglutination tests with S. pyogenes aureus were less positive and following more similar treatments agglutination tests became entirely negative with S. pyogenes aureus; at the same time the cutaneous reaction became negative with the protein of S. pyogenes albus and it was less positive with the protein of S. pyogenes aureus.

The other three cases are so similar that they may be discussed together. Case M. M. gave positive cutaneous reactions with S. pyogenes aureus protein and the serum agglutinated this organism in a dilution of 1–200. During treatment with S. pyogenes aureus vaccines the cutaneous reaction and agglutination tests became negative and asthma was practically relieved. Case P. McL. gave positive cutaneous reactions with S. pyogenes aureus protein and his serum agglutinated this organism in dilutions of 1–150 and 1–200. During three months' treatment with S. pyogenes

aureus vaccine agglutination tests became negative and asthma was relieved. Case P. J. F. gave slightly positive cutaneous reactions with S. pyogenes aureus protein and his serum agglutinated this organism in a dilution of 1–100. Following vaccine treatments with this organism agglutination tests became negative and asthma was greatly diminished. In none of these cases was S. pyogenes aureus isolated from the sputum, but in one case S. pyogenes albus was isolated.

Therefore these five cases gave positive cutaneous reactions with the protein of S. pyogenes aureus, all sera agglutinated this organism and during vaccine treatments with this organism in four cases and with S. pyogenes albus in one, agglutination tests and cutaneous reactions became negative and asthma was either relieved or greatly diminished. From the sputum of one case was isolated S. pyogenes aureus and from one, S. pyogenes albus; neither organism was isolated from the sputa of the other cases. In the one case which was treated with S. pyogenes albus vaccines, agglutination tests became negative with S. pyogenes aureus. Asthma improves rapidly and the cutaneous reaction and agglutination tests return to normal following proper vaccine treatment.

Protocol II.

Positive agglutination tests between S. pyogenes aureus and the serum of bronchial asthmatics who give positive cutaneous reactions with the protein of this organism and the effect of treatment with the protein of this organism on the agglutination test.

J. H. N., a man aged 28, has had asthma for five years. On Feb. 23, 1916, cutaneous reactions were positive with the proteins of S. pyogenes aureus and albus, both organisms were isolated from the sputum and agglutination tests with three stock cultures of S. pyogenes aureus were positive in the following dilutions, — No. 1 = 1–200, No. 2 = 1–200, and No. 4 = 1–150. The patient was treated with six subcutaneous injections of S. pyogenes aureus protein (for method of preparation see Study III.) in gradually increasing amounts of from one to three milligrams at each dose. During these treatments asthma gradually diminished, but the

agglutination tests were practically the same as before, and they were positive in the following dilutions, — No. 1 = 1–200, No. 2 = 1–150, and No. 4 = 1–150. During four more similar treatments the patient became practically free from asthma, the cutaneous reaction became practically negative, but the agglutination tests were practically the same as before and they were positive in the following dilutions, — No. 1 = 1–150, No. 2 = 1–150, and No. 4 = 1–150.

A. D., a woman aged 31, has had asthma for eighteen months. On Feb. 25, 1916, cutaneous reactions with S. pyogenes aureus protein were positive, from the nasal secretions were isolated S. pyogenes aureus and albus, but neither were isolated from the sputum and agglutination tests with four stock cultures of S. pyogenes aureus were positive in the following dilutions, — No. 1 = 1–200, No. 2 = 1–100, No. 4 = 1–100, and No. 5 = 1–150. The patient was treated with three subcutaneous injections of S. pyogenes aureus protein, each dose varying between one and two milligrams. Following these treatments agglutination tests were positive in the following dilutions, — No. 1 = 1–200, No. 2 = 1–100, No. 4 = 1–50, and No. 5 = 1–100. Three more similar treatments were given, during which time the patient became free of asthma. Agglutination tests were now positive in the following dilutions, — No. 1 = 1–200, No. 2 = 1–150, No. 4 = 1–150, and No. 5 = 1–100. Three more similar treatments were given with the protein in amounts varying between two and three milligrams. Following these treatments agglutination tests were positive in the following dilutions, — No. 1 = 1–150, No. 2 = 1–200, No. 4 = 1–100, and No. 5 = 1–100. The patient has been free from asthma for two months.

These two patients were treated with the protein of S. pyogenes aureus instead of with the whole killed bacteria. These two cases are so nearly identical that a discussion of one will answer for both. The first case gave positive cutaneous reactions with the protein of S. pyogenes aureus and albus, his sputum contained both organisms and his serum agglutinated S. pyogenes aureus in high dilutions. During six subcutaneous injections with the protein of S. pyogenes aureus asthma was greatly diminished, but the agglutination tests were as positive as before treatment. Following four more similar treatments, the patient was practically relieved of asthma, the cutaneous reaction was almost negative, but agglutination tests were positive in practically the same dilutions as before treatment. As was presented in Study XII., the serum of both of these patients gave negative complement fixation and positive precipitin reactions with S. pyogenes aureus antigen previous to treatment, and after treatment both of these reactions were negative.

Thus these patients are similar to those presented in the preceding protocol in that they gave positive cutaneous reactions and their sera agglutinated S. pyogenes aureus. The

method of treatment, however, and the results of treatment on the agglutination test are dissimilar in the two protocols. Following treatment with vaccines, agglutination tests became negative, whereas following treatment with the protein of the same organism agglutination tests were not changed, although each method of treatment had a similar beneficial effect on the asthma and on the cutaneous reactions.

Protocol III.

Positive agglutination tests between S. pyogenes aureus and the serum of bronchial asthmatics who give negative cutaneous reactions with the protein of this organism and the effect of vaccine treatment with this organism on the agglutination test.

J. C., a boy aged 13, has had asthma for years. On Sept. 30, 1916, cutaneous reactions were negative with bacterial proteins, from the sputum were isolated S. pyogenes aureus and albus, and agglutination tests with three stock cultures of S. pyogenes aureus were positive in the following dilutions, — No. 1 = 1–150, No. 4 = 1–200, and No. 8 = 1–200. The patient was treated at weekly intervals for a period of three months with S. pyogenes aureus vaccines in amounts varying between 200 and 400 million. Following this treatment (on Dec. 12, 1916) agglutination tests were negative with the three stock cultures mentioned above and with the cultures which were isolated from the patient's sputum. The patient had no asthma after the fourth treatment.

C. G., a man aged 41, has had asthma for four years. On Sept. 20, 1916, cutaneous reactions were negative with bacterial proteins and agglutination tests with four stock cultures of S. pyogenes aureus were positive in the following dilutions, — No. 2 = 1–100, No. 5 = 1–100, No. 9 = 1–200, and No. 13 = 1-200. Patient was given S. pyogenes aureus vaccines at irregular intervals for the next three months. At the end of this time agglutination tests were negative with the stock cultures of S. pyogenes aureus and one month later still, agglutination tests were negative.

V. N., a woman aged 22, has had asthma for two years. On Aug. 10, 1916, cutaneous reactions were negative with bacterial proteins and agglutination tests with six stock cultures of S. pyogenes aureus were positive in the following dilutions, — No. 1 = 1–100, No. 4 = 1–100, No. 7 = 1–50, No. 8 = 1–100, No. 9 = 1–50, and No. 13 = 1–100. The patient was treated at irregular intervals with twelve doses of S. pyogenes aureus vaccine, in amounts varying between 200 and 500 million. The patient had no asthma after the sixth treatment and treatment was discontinued

Nov. 15, 1916. On Jan. 10, 1917, the patient had a return of asthma, but agglutination tests were still negative. Following three vaccine treatments asthma was again relieved. No staphylococci were isolated from the sputum.

B. H., a boy aged 14, has had asthma since eighteen months of age. On Sept. 11, 1916, cutaneous reactions were negative with bacterial proteins, S. pyogenes aureus was isolated from the sputum and agglutination tests with several strains of this organism were positive in a dilution of 1–200. The patient has been treated continuously at weekly intervals with S. pyogenes aureus vaccine in amounts varying between 100 and 700 million; a total of seventeen doses has been given. The patient has had no asthma since the fourth treatment, which was four months ago. On Nov. 7, 1916, following the eighth treatment agglutination tests were negative with the stock cultures of S. pyogenes aureus and also with the culture which was isolated from the patient's sputum. On Jan. 16, 1917, following the seventeenth treatment agglutination tests were still negative.

G. G., a boy aged 13, has had asthma for six years. On Sept. 7. 1916, cutaneous reactions were negative with bacterial proteins, S. pyogenes aureus and albus were isolated from the nasal secretions, but not from the sputum, and agglutination tests with stock cultures of S. pyogenes aureus were positive in a dilution of 1–200. The patient has been treated at weekly intervals with seventeen doses of S. pyogenes aureus vaccine in amounts varying between 100 and 700 million. Since the sixth treatment the patient has had practically no asthma. Following the ninth treatment agglutination tests were negative with the stock cultures of S. pyogenes aureus and with the culture which was isolated from the patient's nasal secretions. After seven more vaccine treatments agglutination tests were still negative.

The cases presented in this protocol are so similar that they may be discussed together. None of the patients gave positive cutaneous reactions with bacterial proteins, but the serum of each patient agglutinated S. pyogenes aureus. Staphylococcus pyogenes aureus seems to have been the cause of asthma in the four youngest patients, since during treatment with vaccines of this organism all asthma ceased and three of these patients have remained free from asthma for five months; the other patient, who had a return of asthma two months after treatment was discontinued, again became free from asthma during a second series of vaccine treatments. Following eight or ten treatments with S. pyogenes aureus vaccine, agglutination tests became negative in

each case and in two of the patients with whom the vaccine treatments were continued for another similar period agglutination tests remained negative. Bacteriological examination of the sputum was made in each case and from one S. pyogenes albus and aureus and from another the latter organism alone were isolated; from the one case on which an examination of the nasal secretion was made both S. pyogenes aureus and albus were isolated.

Thus these cases are similar to the ones presented in Protocol I. in that all agglutinated S. pyogenes aureus, treatment with vaccines of this organism was followed by a negative agglutination test and asthma was relieved following a few vaccine treatments. Staphylococcus pyogenes aureus was isolated from the sputum of some of these cases, but not from the majority of them. These cases are unlike those presented in Protocol I., in that none gave positive cutaneous reactions with bacterial proteins, whereas all patients presented in the former protocol did give positive cutaneous reactions with S. pyogenes aureus proteins.

Protocol IV.

Positive agglutination tests between S. pyogenes aureus and the serum of bronchial asthmatics which give positive cutaneous reactions with proteins other than bacterial.

C. N. E., a man aged 25, has had asthma for twenty years. Cutaneous reactions were positive with proteins from wheat and horse dandruff, but they were negative with bacterial proteins. His serum agglutinated four stock cultures of S. pyogenes aureus in the following dilutions, — No. 1 = 1–100, No. 4 = 1–100, No. 5 = 1–50, and No. 7 = 1–50. Following ten weekly treatments with subcutaneous injections of horse dandruff proteins, agglutination tests were positive in the following dilutions,— No. 1 = 1–50, No 4 = 1–50, No. 5 = 1–50, and No. 7 = 1–100. During the next four months, treatment was given with the wheat proteins in a similar manner and at the end of this time agglutination tests were negative with S. pyogenes aureus. Asthma was relieved.

G. V. Y., a woman aged 28, has had asthma for six years. Cutaneous reactions were positive with cat hair proteins, but negative with bacterial proteins. Her serum agglutinated six stock cultures of S. pyogenes

aureus in the following dilutions, — No. 1 = 1–50, No. 2 = 1–150, No. 4 = 1–150, No. 5 = 1–100, No. 7 = 1–100, and No. 8 = 1–50. During the next six months the patient was treated with subcutaneous injections of cat hair protein and at the end of this time agglutination tests were negative. Asthma was relieved.

D. G., a woman aged 20, has had asthma for two years. Cutaneous reactions were positive with horse dandruff proteins, but negative with bacterial proteins. Agglutination tests were positive with S. pyogenes aureus in the following dilutions, — No. 1 = 1–150 and No. 2 = 1–50. Following four injections with horse dandruff protein, agglutination tests were positive with only one strain and in a dilution of only 1–50. Following a few more similar treatments, agglutination tests were entirely negative, and asthma was relieved.

These patients are presented as controls for those in the preceding protocols. They gave positive cutaneous reactions with proteins other than bacterial ones and their serum agglutinated S. pyogenes aureus. During treatment with subcutaneous injections of the protein to which they were sensitive, asthma was relieved and agglutination tests became negative with S. pyogenes aureus. Case F. S. E. which was presented in Protocol I. is similar to these cases.

Probably agglutination tests in these cases would have become negative without any treatment and one is not justified in saying that they prove that injections with a foreign protein had anything to do with making the agglutinations negative. The same reasoning must be used in the cases which were presented in former protocols and thus there is no positive evidence that treatment with vaccines played any part in making agglutination tests negative in the former protocols. There are, however, differences between the cases which are presented in the last protocol and those presented in former protocols, and these differences favor the assumption that vaccines of S. pyogenes aureus had something to do with making agglutination tests negative with this organism. In the former protocols the serum of each patient agglutinated S. pyogenes aureus in high dilutions and each of the several strains of the organism were agglutinated in the same dilution; in other words, these were good, strong, positive agglutination tests. The patients who

were presented in the last protocol required a much stronger dilution of serum for agglutination and there was much variation between the dilutions which were positive with the several strains of the organism: these tests were not strongly positive and one would not feel sure that they could be repeated with similar results.

In order to make this paper complete, it should be stated that six patients were studied who gave positive cutaneous reactions with S. pyogenes aureus protein, but their sera did not agglutinate this organism and it was not isolated from their sputa. Three of these patients were treated with S. pyogenes aureus vaccines and their asthma was relieved. The other three were treated with the other proteins to which they were sensitive. It might be stated that S. pyogenes aureus was isolated from the sputum and nasal secretions of patients who failed to give positive cutaneous reactions with the protein of this organism and whose sera failed to agglutinate strains of this organism.

SUMMARY. — This paper is based chiefly on thirty patients with bronchial asthma whose sera agglutinated S. pyogenes aureus. Many of these patients gave a positive cutaneous reaction with the protein of S. pyogenes aureus and in a few of them this organism or S. pyogenes albus was isolated from the sputum or nasal secretions. Those patients who gave positive cutaneous reactions with S. pyogenes aureus protein and whose serum agglutinated this organism were greatly improved or were relieved of asthma during treatment with vaccines of this organism and both tests became negative. Those patients in whom both of these tests were positive and who were treated with the protein of this organism were likewise greatly improved or relieved of asthma, but agglutination tests were not changed. A few patients who failed to give a positive cutaneous reaction with any protein whatsoever did give a positive agglutination between their serum and S. pyogenes aureus and this was the only indication we

had as to the possible cause of asthma in these cases; during treatment with vaccines of this organism, asthma was relieved and agglutination tests became negative. From the sputum and nasal secretions of this latter group of cases S. pyogenes aureus and albus were isolated in a few instances. A few patients who did not give positive cutaneous reactions with bacterial proteins, but did give positive cutaneous reactions with other proteins, also agglutinated S. pyogenes aureus and during treatment with subcutaneous injections of the proteins to which they were sensitive asthma was relieved and agglutination tests became negative. In the latter group of cases agglutination tests were not positive with a high titer of serum, in fact these tests were so variable with different strains of the organism that they can really be called not more than suspiciously positive. Nevertheless the fact that agglutination tests did become negative in these latter cases does not permit us to conclude that vaccine treatment played any part in making agglutination tests negative with the patients mentioned earlier in this summary. Several patients were studied who gave positive cutaneous reactions with the protein of S. pyogenes aureus, but their sera failed to agglutinate this organism and from their sputa the organism was not isolated. Furthermore, S. pyogenes aureus and albus were isolated from the sputum or the nasal secretions or from both in some patients who failed to give positive cutaneous reactions with the protein of these organisms and whose serum failed to agglutinate them. Therefore there seems to be no relationship between the positive cutaneous reaction, the serum agglutinatian test, and the isolation of S. pyogenes aureus and albus from the sputum and nasal secretions of patients with bronchial asthma. The cutaneous reaction has proved to be the safest and best guide in the cause and treatment of bronchial asthma and in a few instances serum agglutination tests have been the only guide in the cause and treatment of this disease. The isolation of bacteria from the sputum and nasal secretions bears no fixed relation to either of these tests.

CONCLUSIONS.

The cutaneous reaction has proved to be the safest and best test for determining the bacterial cause of bronchial asthma.

When cutaneous reactions have failed, serum agglutination tests have been of value in a few cases in determining S. pyogenes aureus as the cause of bronchial asthma. This test is of no value with S. pyogenes albus since no serum agglutinated this organism.

There is no fixed relationship between the cutaneous reaction, serum agglutination tests and the isolation of S. pyogenes aureus and albus from the sputum and nasal secretions of patients with bronchial asthma.

Vaccine treatments with S. pyogenes aureus and albus do not increase the titer of a serum which agglutinates these organisms; in fact agglutination tests become negative during treatment with vaccines from them.

NEUROBLASTOMA.*

WITH REPORT OF A CASE.

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Those primary tumors of the adrenal capsule, whose origin is ascribed to the nervous elements of the sympathetic system, form a group that, both on account of their rarity and on account of the histogenetic problems involved, has furnished an interesting chapter in the literature of pathology. In view of the small number of cases on which the recognition of this class of neoplasms is based, the report of another typical instance is of value; to that end this contribution is made.

According to the generally accepted opinion, these tumors arise from certain embryonic cells of the neuroectodermal system. These cells migrate ventrally in early fetal life from the anlage of the spinal cord to form the sympathetic chains and their paraganglia. The penetration of numbers of these cells into the already formed cortex of the adrenal gives origin to the medulla of that organ. In this stage of development, the cells are of an undifferentiated but characteristic type, the sympathetic neuroblast. Later, in the adrenal and the other paraganglia, the differentiation into chromaffine cells and ganglion cells takes place. The cells of the highly malignant sympathetic *Neuroblastoma* correspond to the cells in the neuroblastic stage. The cells of the *Paraganglioma* and *Ganglioneuroma* correspond to the differentiated forms. The former is the chromaffine-cell tumor, and the latter, the ganglion-cell tumor. The histogenetic relationship of these three neoplasms is now established, largely by the recent recognition of groups of undifferentiated cells in tumors of mature type, and of

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mature cells in tumors of embryonic type. Neuroblastoma and ganglioneuroma represent, therefore, the two extremes of a process of differentiation which may be considered as taking place, in some instances at least, in the course of growth of an individual tumor (Pick[35]). In other instances the degree of differentiation of the tumor may be the result of its origin at a particular stage of normal development of the sympathetic. These tumors may occur wherever the nervous elements of the sympathetic system occur.

It must be remembered, furthermore, that these tumors are related to the corresponding tumors of the central nervous system through the parent cell of the whole group, namely, the embryonic neurocyte of the neuroectodermal system before even that grade of differentiation has been reached which entails the migration of the sympathetic neuroblast. From these cells of the central nervous system are derived the neurocytoma of Marchand (embryonic), and the true neuroma of Virchow (mature). The glioma is believed to represent the product of another specialization of this parent cell.

The tumor which I have had the opportunity to study falls unmistakably in the group of the embryonic undifferentiated type, neuroblastoma.

Since ganglioneuroma and neuroblastoma represent different stages in the development of one tissue there is difficulty in a discussion of either tumor separate from the other. However, the clinical and pathological characteristics of the relatively pure neuroblastoma are sufficiently individual to warrant the attempt in a report of this kind.

Historical. — The history of neuroblastoma may be divided into two periods:

(1) An early period before the tumor was recognized as composed of tissue of nervous origin.

(2) A later period, characterized by general agreement in recognition of the tumor as of nerve tissue, but marked by discussion relative to the particular element of nerve

tissue involved. The two schools have championed, respectively, the glia theory and the embryonic neuroblast theory. In the last few years the latter theory finds itself predominant. These periods are not sharply marked off from each other.

The literature is rich in case reports of congenital malignant tumors of the adrenal associated with liver metastases. Before the suggestion of Virchow [1] in 1864 that these might be of nerve-tissue origin (gliomata) they had probably all been classed as sarcomata of various types. Even after this remarkable generalization, the diagnosis of sarcoma was frequent. In 1885 Dalton [3] published the first case so completely described from the histological aspect as to enable us to be certain that the tumor was neuroblastoma. He regarded it as a lymphosarcoma. Marchand,[6] six years later, reported the second unmistakable case of the disease and the first case to be recognized as a nerve-tissue neoplasm. He compared the cells to the neuroblasts of the fetal sympathetic ganglion, from which we now believe the growth takes origin, and suggested the development of mature ganglion cells from the tumor cell. From Marchand, 1891, to Amberg,[14] 1904, with the exception of a case reported by Orr [11] in 1900, no undoubted instances of the tumor can be found in the literature. A number of cases of similar new growths were reported, but the accurate histological picture is lacking from all such reports. There must, however, have been a number of cases recognized, for Kretz,[13] writing before Amberg, makes the suggestion again that the tumors are derived from the fetal sympathetic system. Amberg, like Dalton, failed to recognize the nerve-tissue character of the tumor.

In 1905 appeared one of the landmarks of the discussion. Küster,[17] reverting to Virchow's suggestion and supported by Ribbert, interpreted his tumor as a glioma. In spite of Wiesel's [18] warning, which appeared shortly afterward and reiterated Marchand's and Kretz's comparisons of the cells to those of fetal sympathetic structures, this report of Küster had a profound influence on the attitude of observers; it

was felt that the gliomatous character of the tumor was fairly established. Thus two years after Küster's paper, Lapointe and Lecène,[19] after submitting the tissue of their quite characteristic case to Ribbert, followed Küster's example in the diagnosis of glioma. Küster was the first to use the term "rosette," which has since been uniformly applied to the characteristic element of the microscopical architecture. Alezais and Imbert[21] in 1907 added a new interest to the discussion by reporting a similar tumor occurring in the region of the coccygeal body. In 1908 for the last time the diagnosis of sarcoma was made on a tumor that was unmistakably a neuroblastoma; this was the case reported by Tileston and Wolbach.[23] During the next year, Schilder[27] published a comprehensive paper with the report of a case, supporting the glial theory of origin.

The year 1910 was signalized by the first authoritative support of the neuroblast theory; J. H. Wright,[28] in a widely-mentioned paper, crystallized sentiment in favor of the present belief. He was the first to use the term *neuroblastoma*, but he did not make the distinction between neuroblastoma and neurocytoma that recent authors have developed.

Since this date, the evidence in support of the theory has been cumulative, emphasizing in more than one way the embryonic relationships of the growth. Scholarly papers by Pick (1912),[35] Landau (1912),[36] Herxheimer (1913),[37] and Wahl (1914),[45] and a number of shorter case reports demonstrate the following facts:

(1) That in the typical neuroblastoma there may occur mature ganglion cells, and that the transitions from the neuroblast to the older type may be traced (Pick,[35] Landau,[36] Martius,[38] Wahl,[45] Dunn[48]).

(2) That, in such instances, the development of a mature nerve associated with the ganglion cell may also be traced (Pick,[35] Wahl[45]).

(3) That in a ganglioneuroma there may occur islands of neuroblastomatous tissue (Pick and Bielschowsky,[30] Peters,[39] Freund,[40] Dunn,[48] Monro and Dunn,[46] Robertson[49]).

(4) That a ganglioneuroma may metastasize widely, all

of the metastases being characteristic of neuroblastoma (Monro and Dunn [46]).

(5) That there may be two distinct united tumors, one a ganglioneuroma, the other a neuroblastoma (Martius [38]).

(6) That the more mature the tumor tissue appears under the microscope, the older the patient and the less malignant the tumor tend to be (Landau [36]).

One must add to these facts the already mentioned similarity of the microscopical picture of neuroblastoma to that of the anlage of the sympathetic system. This has been convincingly demonstrated by the photomicrographs of Wright,[28] Landau,[36] Dunn,[48] and Wahl [45] (see Fig. 16).

On these observations the present conception as outlined in the earlier portion of this paper is built up.

With the exception of Glomset (1915),[47] whose statements do not seriously menace either these observations or the conclusions, all the recent writers on this subject agree.

The questions of nomenclature and classification have interested many authors. It is evident that there is no sharp dividing line between ganglioneuroma on the one hand and neuroblastoma on the other; the two types melt into each other through intermediate mixed types. One is forced to the conclusion, moreover, that most instances of the two extreme types are also mixed to a greater or less degree. In other words, even the extreme types have common characteristics that will not warrant their designation as separate entities. For the sake of convenience in summarizing the literature and in consideration of the great differences between the two types irrespective of their similarities, the use of the two terms, as in this report, seems justified. Robertson (1915) [49] has attempted to solve the problem by the addition of another class, ganglioneuroblastoma, to include those mixed types with a large amount of embryonic tissue. This suggestion, of course, does not obviate the fundamental impossibility of a satisfactory classification of these tumors. It can be justified, therefore, only on the ground that it offers a convenient and readily applicable term. To my mind the

suggestion puts forward a third term, without a sharply-defined application, to add to the other two of equally ill-defined meaning; it multiplies words without making the classification more sharply cut.

Case Report.

M. H., white male, æt. eleven months.

Complaint: Swelling of abdomen, noticed by parents.

Family history: Father and mother are living and well. One sister, seven years old, is living and well. Patient has no other brothers or sisters. History of both sides of the family for two generations reveals unusual longevity. There is no suggestion of malignant tumor or hereditary defect in the family.

Past history: The child was born spontaneously at term by a frank breech presentation. Duration of labor was seventeen and one-half hours. Child did not breathe for some time, but respiration was finally established after considerable effort. Child has been breast-fed since birth. Nineteen days after birth the mother noticed a lump in the right side of the neck; this has decreased in size, but has not disappeared. Otherwise there has been nothing abnormal.

Present illness: At an undetermined period, the mother noticed swelling of the abdomen, which has been progressive. It has not been accompanied by marked constitutional symptoms.

Physical examination: Well-developed and well-nourished child. Examination unimportant except for the presence of abdominal and cervical tumors. In the right side of the neck along the sterno-mastoid muscle are two or three nodes about the size of a small pecan, smooth in outline, fairly movable. The skin over them is free and of normal color and temperature.

In the right side of the abdomen, filling the flank, is a round, smooth tumor, the size of a fist, freely movable, dull to percussion. It feels somewhat like a tense cyst.

X-ray examination: Plates revealed a rounded shadow in the abdomen corresponding to the tumor removed at operation.

Pre-operative diagnosis: Cystic kidney?

Operation: The tumor was removed by a laparotomy on Oct. 14, 1916, by Dr. Willard Bartlett of this city, to whom I am indebted for the specimen and clinical records. A right rectus incision was made, and the tumor was exposed after incision of the posterior peritoneum. The growth occupied the entire right flank, was encapsulated and somewhat movable. It lay to the right of the ascending colon. From the posterior mesial surface arose a poorly-defined pedicle containing one or two large vessels. These were quickly clamped and divided and the tumor removed. The ureter was looked for but not seen. The pedicle was then tied with catgut. The condition of the child was such that a complete exploration

was not deemed advisable; the abdomen was therefore closed without an accurate determination of the origin and relations of the tumor. The liver appeared normal to hasty inspection. Closure was made without drainage.

Subsequent course: The patient made a satisfactory operative recovery. On Nov. 1, 1916, the patient weighed 21 pounds; on Dec. 1, 1916, 22 pounds; on Dec. 27, 1916, 23¼ pounds. (All weights have been measured by the mother.) In view of the child's tremendous handicap, this progress must be considered altogether satisfactory. Eruption of teeth was taking place throughout this period. Enunciation of single words has begun. Child can walk alone. Mother writes (Dec. 27, 1916): "Eats hearty, had no fever, no stomach disorder, cheeks look rosy, lips are more red. His appetite is very good, his stools look fine. I often look for a lump in his abdomen, but can't find anything so far. His neck is just the same."

Pathological report: The tissue was brought to the surgical laboratory of the Washington University Medical School about three hours after removal. In the fresh condition the mass measured 13 by 11 by 8 centimeters, and weighed 470 grams. The tumor (Fig. 1) is in general oval, and of regular contour. In one of the broad aspects, however, there is a marked rounded depression involving almost the entire surface; the bottom of the depression is approximately 1 centimeter below the level of the plane tangential to its rim. A greatly distended adrenal may be imagined to assume this shape.

The tumor is completely encapsulated by a fibrous membrane, from which the ends of several small severed vessels protrude. On the surface of the capsule runs a network of thin-walled blood vessels, the largest 4 millimeters across, emerging from and penetrating the substance of the tumor in no regular manner. These vessels run often in shallow sulci dividing well-marked surface nodules from 1 to 2 centimeters in diameter. The surface color is grayish pink with large irregular areas of intra- or sub-capsular hemorrhage. The consistency of the mass is generally somewhat soft, but the nodules themselves are more firm and can be made to move independently to a limited extent in the general semi-fluctuant substance.

On section the tumor (Fig. 2) presents a striking picture. The surface nodules are seen to represent spherical and irregular subdivisions of the tissue, each of which is more or less completely encapsulated by a definite fibrous membrane. The cut surface presents largely a pure white, glistening, glairy, soft, homogeneous substance from which a pearly juice may be expressed. This background is splashed irregularly with brilliant colors ranging from bright orange through red to black. One of the limited nodules is entirely of a brick-red color. The colored areas are less glistening, and in places appear dry and granular. Spots of excessive softness occur, suggesting necrosis. The capsule of the tumor is seen to be dense and of slightly varying thickness.

The tumor was placed at once in Kaiserling fluid No. 1, and, after two days of hardening, blocks were cut, imbedded in paraffine and stained with hematoxylin and eosin. The gross specimen in the meantime had been carried on through the Kaiserling process. When it became evident from a study of these sections that further examination was imperative, the tissue was removed from Kaiserling solution No. 3, and a wedge was sliced from the cut surface including practically all of an equatorial section in the long diameter. This tissue was washed thoroughly, cut into blocks, and imbedded in celloidin. Stains employed were hematoxylin and eosin, Van Gieson, phosphotungstic acid hematoxylin, and Bielschowsky's silver impregnation.

Histology: The stroma of the tumor consists of fine connective tissue vascular septa, marking off round to irregular alveoli, which contain the tumor tissue proper. These alveoli average in size somewhat less than the field of the No. 5 Leitz objective with No. 4 eyepiece.

The connective tissue boundaries of these units merge into a few heavier septa that surround the lobules noted in the gross. The capsules of the latter fuse with the connective tissue of the general capsule. Nested within the general capsule may be found compressed groups and cords of cells that in places are typical of the cortical epithelium of the adrenal (Figs. 14, 15).

The tumor tissue consists of two distinct varieties of cells, transitional cell forms, and an intercellular substance (Figs. 5, 6).

A cell a little larger than a lymphocyte with a minute amount of cytoplasm is overwhelmingly the most frequent cell. The nucleus is round to pyriform and contains a large amount of dark-staining chromatin. The size of this cell varies within small limits. Where the cytoplasm can be observed it is slightly basophilic and finely granular.

There is present also a cell with a large pale nucleus and slightly acidophilic, pale, abundant cytoplasm that often takes a stellate or elongated form. It occurs in relatively small numbers. Rarely it is multinucleated. It suggests the ganglion cell, but it is certainly not typical of the mature form.

Cells that may be regarded as transitional between these two distinct types are found in small numbers.

Among these cellular elements lies the characteristic intercellular substance, a very fine network of minute fibrils taking a pale pink stain. The cell bodies are often in close relation to this substance; with the oil-immersion objective it is possible to trace the fusion of the fibrils with the cytoplasm.

Two main varieties of architecture are seen. The major part of the tumor is made up of dense masses of the more prevalent cell, filling the alveoli and producing a picture that resembles a sarcoma of the small, round cell type. In these regions the larger pale cell is not found and the intercellular substance is often meager (Fig. 3).

Standing out in sharp contrast to this background are less cellular areas in which the characteristic structures of the neoplasm occur. These areas are found scattered in the midst of the dense structure, or in zones one to two centimeters in diameter. They merge with no line of demarcation into the cellular structure, the transition occurring often within the limits of a single alveolus (Fig. 4).

In these portions of the tumor the intercellular substance is abundant and the large cell and transitional form appear frequently. Here also several quite characteristic arrangements of the small cell and the fibrillar substance may be found.

(1) The tumor cells are occasionally clumped together in spherical agglomerations of perhaps four to a dozen cells. There is no syncytial merging of the cytoplasm in these structures (Fig. 7).

(2) An oval ring of cells occurs about a central mass of fibers more dense than is the network elsewhere. The cells are two to three rows deep. The outer ring is not marked off from the surrounding tissue except by its cellular richness. In these structures, which are the typical "rosettes" of the literature, the nuclei tend to be pyriform with the long axes radially directed. The rosettes occur rather sparsely (Fig. 8).

(3) In the third form the cells are massed at one or both ends of a mass of intercellular substance, the fibrils of which tend to run parallel or to spread fanwise towards the area of

greatest cell concentration. This mass of fibrils may be practically cell-free, or it may be dotted with nuclei in parallel chains. The size is very variable (Figs. 11, 12).

(4) Forms intermediate between the first and second and the second and third of these forms may also be observed (Figs. 9, 10).

It will bear repetition that these arrangements are rare relative to the mass of uncharacteristic tissue.

There is extensive interstitial hemorrhage in both stroma and tumor tissue (Fig. 13). In places are collections of endothelial cells containing yellow to orange pigment granules. Small zones of necrosis occur with infiltration of inflammatory cells.

With the phosphotungstic acid hematoxylin stain, the fibrils take a blue color.

Impregnation by Bielschowsky's method is not satisfactory. A few fibrils are deeply stained, but it is difficult to rule out the possibility that these few represent the fine collagen strands that occasionally are seen in the alveoli.

Van Gieson's stain demonstrates that the collagen is almost entirely limited to the stroma, although a few fine fibers, usually accompanying capillaries, rarely invade the alveolar cell-masses. The intercellular substance of the tumor takes a brown coloring.

Diagnosis: Neuroblastoma, primary in the medulla of the adrenal.

Discussion. — The demonstration of adrenal cortical cells in the capsule proves the site of this tumor which the operation failed to show. The medulla of the adrenal is the most frequent site of neuroblastoma (see table), but cases have also been reported from the sympathetic chains of the abdomen (Schilder,[27] Wright,[28] Landau,[36] Anitschkow[41]), and neck (Martius[38]), and from the uterus (Pick[35]). A noteworthy fact is the incidence, frequently, of the neoplasm simultaneously in both adrenals.

The age of the patient is characteristic (see table). Sex has no relation to the occurrence of the tumor.

When the tumor occurs in the adrenal it is practically

always encapsulated with the stretched capsule and atrophied cortex of that organ. Many observers mention the striking "respect" that the tumor has for nearby structures; it is often adherent to a compressed kidney or liver, but rarely fuses with it. Quite different, however, is the case when the tumor occurs in the other sympathetic structures, for then the invasive malignancy is predominant. Anitschkow's [41] case had even invaded the spinal canal, remaining extradural.

Metastasis, however, even from the encapsulated adrenal growth, is in most instances extensive and the metastases rapidly progressive. The liver is the favorite site, with regional lymph glands and bones, especially of the skull, not infrequently involved. Several cases of more generalized metastases are recorded, but even in these instances the spread is somewhat selective. The extensive liver metastases of a type ordinarily associated with blood dissemination may, perhaps, be explained, as Landau [36] suggests, by the patency of the foramen ovale, which was present in his case and is recorded in other cases.

The present case is quite characteristic in its possession of a capsule, but it apparently is not similar to the majority of those recorded in regard to metastases. Neither at operation nor since has there been evidence of any secondary growths. The tumors in the neck cannot be considered as metastases, for they are regressive. Moreover, the child has been gaining in weight and strength progressively. Nevertheless, the tumor is, as will be shown below, distinctly of an undifferentiated type. On a theoretical basis, according to Landau's generalization, one would expect it to be extremely malignant.

The description of this tumor in the gross, as recorded above, can be made only in terms that have been used in describing the earlier recorded cases; in other words, the tissue possesses a characteristic appearance.

The microscopical picture is equally characteristic, agreeing as well with the photomicrographs to be found in the literature. The published descriptions of these tumors are

in striking accord; the histological picture is an unmistakably individual one.

This particular tumor contains a large amount of the sarcoma-like tissue that Landau[36] believes to be the most undifferentiated form, tissue in which the intercellular fibrils can often barely be distinguished. It contains the earliest form of ganglion cell, but no mature ganglion cells. Moreover, the development of nerve fibers can only be partially followed out (Figs. 7–12). This process is described by Pick[35] and Wahl[45] as passing through the several stages of the rosette (as described in this tumor) into still older stages that do not occur here. From these facts, it is clear that the tumor is relatively undifferentiated.

The staining reactions, with the exception of that by Bielschowsky's method, are similar to those recorded in earlier cases. Herxheimer[37] and Robertson[49] believe that a successful silver impregnation is necessary for the establishment of a diagnosis. This method is notoriously difficult, the difficulty being increased in the case of embryonic or pathological tissue. In view of the very definite microscopical picture, as well as the other facts already mentioned, it seems to me that the diagnosis can be made without this desirable but unnecessary aid. It must be remembered that Herxheimer wrote before several of the most instructive cases had been reported.

This case is distinctive among undifferentiated tumors in one respect that deserves mention. It is the first case successfully operated upon. Although theoretically one cannot avoid the feeling that evidence of metastasis may occur, yet two and one-half months after operation the child's health continues to improve. All other cases that have come to operation have died during or shortly after the operative procedure.

ADDENDUM.

Tabulations of cases of neuroblastoma occurring in the literature have been published in two or three of the later studies of the disease, but these tables all lose value through the inclusion of some of which the diagnosis cannot be unquestionably established from the published reports.

There are a certain number which are unmistakably recognizable. On these practically all authors agree. To the study of these cases the time of investigators can more profitably be applied than to the study of a large number of which the reports do not permit more than a presumptive diagnosis. For this reason there is appended a tabulation of the relatively few cases of positive diagnosis under the heading of *Cases of Neuroblastoma;* and below are listed those which may or may not be examples of this condition under the heading *Doubtful Cases.* It is to be remembered that this classification is based with one exception on the completeness and accuracy of the published case reports, and that the material is limited to the tumors that are distinctly of the neuroblastoma type, as opposed to mixed forms.

CASES OF NEUROBLASTOMA. (See accompanying Table.)

(1) Dalton, 1885.[3] In this report occurs the first description of the rosette: " . . . the cells are mingled with a peculiar finely-granular and, in places, perhaps slightly fibrillated substance. In some places the round cells are scattered uniformly through this material; in others they are arranged around the borders of a small mass of it, something like the nuclei of a giant-cell."

(2) Marchand, 1891.[6] The author makes no diagnosis, but from his comparison of the cells of the tumor to those of the fetal sympathetic, it is evident that he was close to the correct conception of the origin of the tumor.

(3) Orr, 1900.[11] Wahl,[45] in his table of cases, does not refer to this instance. However, the report is complete, including the earliest photomicrographs of the tumor to be found; and other authors (Pick,[35] Herxheimer[37]) agree on the diagnosis. There seems to be no question of the identity of the tumor.

(4) Amberg, 1904.[14] This is a typical case. The author, like Marchand, compares the cells to those of the fetal adrenal, but also does not commit himself to a diagnosis.

(5) Richards, 1905.[16]

(6) Küster, 1905.[17] In this report the term "rosette" is first applied.

(7) Lapointe and Lecène, 1907.[19]

(8) Alezais and Imbert, 1907.[21] This case has been accepted by all authors as a typical case of neuroblastoma. From the description, however, it should perhaps be put down as a ganglioneuroma. On account of the close relationship of these two tumors, however, the diagnosis cannot be absolute. For this reason, I have included the case between brackets.

(9) Tileston and Wolbach, 1908.[23] Photomicrographs establish the diagnosis beyond question.

(10) Hecht, 1909.[25] This case also is bracketed. The histological description is typical except that no rosettes are described. Pick, Herxheimer, and Wahl consider the case established.

(11) Schilder, 1909.[27] This report is the most recent to make a definite diagnosis of glioma. The case is undoubtedly a neuroblastoma.

(12) Wright, 1910.[28] Case 1. Excellent photomicrographs of this and the succeeding case make the diagnosis certain.

(13) Wright, 1910.[28] Case 4.

(14) Wolbach, 1911. This case is the exception referred to. There is no complete report to be found in the literature. It is mentioned by Pick,[35] the tissue having been sent to him, and he agrees in Wolbach's diagnosis of neuroblastoma. Through the courtesy of Dr. Wolbach I have been enabled to study a section from a submaxillary metastasis of this tumor. The picture is characteristic although no mature rosettes are seen; these, of course, may have been present in the primary growth. In spite of the unique site, the diagnosis may be considered established.

(15) Pick, 1912.[35] An interesting case from the origin in the uterus, it is undoubtedly a neuroblastoma.

(16) Landau, 1912.[36] Case 1. The author notes the transition of rosettes to fiber bundles as distinctive of neuroblastoma from glioma.

(17) Landau, 1912.[36] Case 2.

(18) Landau, 1912.[36] Case 3. This is a case of very undifferentiated type. The rosettes occur only in the earliest form.

(19) Herxheimer, 1913.[37]

(20) Martius, 1913.[38] This tumor is interesting in the fact that two growths of different nature attached to each other occurred in the cervical sympathetic. One of these was a neuroblastoma, the other a ganglioneuroma.

(21) Anitschkow, 1913.[41] In this otherwise typical tumor occurred rosettes with lumina, of which drawings are given. This is a unique finding. Several observers have described the tendency of the contents of the alveolar units in these tumors to shrink away from the stroma during the process of fixing and imbedding. Such distortion has occurred in the tumor reported here (Fig. 6). It is possible, of course, that these lumina represent some such artefact peculiar to this case. Anitschkow himself believes them a further indication of the close genetic relationship of glia cell and neuroblast through the parent neuroectodermal element. Under such a conception, the cells of this tumor had retained, in abnormal differentiation, a character that the parent cell ordinarily transmits only to its glial offspring. In the presence of typical rosettes and other characteristic features, these atypical findings do not invalidate the diagnosis.

(22) Wahl, 1914.[45] The process of differentiation from neuroblast to ganglion cell and from the earliest rosette to a nerve-like body are traceable in this tumor.

(23) Dunn, 1915.[48] This case is remarkable in the age of the patient, (14 years). It presents otherwise a characteristic picture.

(24) Glomset, 1915.[47]

(25) Lehman, 1917.

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REPORTED CASES OF NEUROBLASTOMA (SEE ACCOMPANYING REMARKS).

	Date.	Author	Sex.	Age.	Site.	Size.	Metastases.	Gross Appearance.	Microscopic Appearance.	Pure or Mixed with Mature Cells	Diagnosis.	Illustrations.
(1)	1885	Dalton.	M.	6 weeks.	Left adrenal.	Hen's egg.	Liver.	Typical.	Typical.	No larger cells described.	Lympho-sarcoma	None.
(2)	1891	Marchand	F.	9 months.	Right adrenal.	Cherry.	None described.	Typical.	Typical.	Larger cells seen; not mature ganglion cells	See remarks.	
(3)	1900......	Orr.	F.	7 weeks.	Both adrenals.	7 ounces.	Liver.	Typical.	Typical.	....	Sarcoma	Photomicrographs.
(4)	1904......	Amberg.	F.	2 months.	Left adrenal.	2.5 x 1.3 x 2.5 cm.	Liver.	Not atypical.	Typical.	"Larger cells seen."	Not made	None.
(5)	1905	Richards.	M.	2 weeks.	Left adrenal.	2 x 1½ in.	Liver.	Typical.	Typical (?).	No larger cells described.	Sarcoma.	None.
(6)	1905......	Kuster.	?	14 weeks.	Both adrenals.	Watch, right. Walnut, left.	Liver.	Typical.	Typical.	No larger cells described	Glioma.	Drawings.
(7)	1907......	Lapointe and Lecène.	F.	19 months.	Left adrenal.	2 fists.	Regional lymph nodes.	Homogeneous surface.	Typical.	No larger cells described.	Glioma.	Drawings.
[(8)	1907......	Alezais and Imbert.	M.	6 years.	Coccygeal gland.	Nut.	None.	Atypical.	Atypical	Many large cells.	"Tumeur parasympathique."	None.]
(9)	1908......	Tileston and Wolbach.	M.	16 months.	Right adrenal.	12 x 8 x 8 cm.	Skull.	Typical.	Typical.	Not mentioned.	Sarcoma.	Photomicrographs.
[(10)	1909......	Hecht.	?	6 years.	Retroperitoneal (?).	?	Liver, lymph nodes, duodenum.	Typical in liver.	Typical. No rosettes described.	Not mentioned.	Glioma sarcomatodes	None.]
(11)	1909......	Schilder.	F.	7 days.	Sympathetic nerve.	13 x 7 x 5 cm.	None.	Typical.	Typical.	Masses of ganglion and chromaffine cells.	Glioma.	Drawings.
(12)	1910......	Wright (1).	M.	Stillborn.	Both adrenals.	?	?	?	Typical.	Not mentioned.	Neurocytoma or neuroblastoma.	Photomicrographs.
(13)	1910......	Wright (4).	F.	16 months.	Retroperitoneal.	?	Skull, mediastinum, pelvis, liver.	?	Typical. No rosettes seen.	Not mentioned.	Neurocytoma or neuroblastoma.	Photomicrographs.
(14)	1911......	Wolbach.	?	3 years.	Cavity of nose.	?	Lymph nodes.	?	"Typical."	Not mentioned.	Ganglioma embryonale sympathicum.	None.
(15)	1912......	Pick.	F.	2½ years.	Uterus.	10 x 8 cm.	Lymph nodes and elsewhere.	Typical.	Typical.	Not mentioned.	Ganglioma embryonale sympathicum.	Drawings.
(16)	1912......	Landau (1).	F.	8 months.	Retroperitoneal.	7 x 7 x 3.5 cm.	Liver.	Typical.	Typical.	None.	Malignant neuroblastoma.	Photomicrographs.
(17)	1912......	Landau (2).	F.	2½ years.	Right adrenal.	Fist.	Lymph nodes, bones, esp. of skull.	Typical.	Typical.	Large pale cells occur.	Malignant neuroblastoma.	Photomicrographs.
(18)	1912......	Landau (3).	F.	Newborn.	Both adrenals.	3.4 x 3.2 x 2.2 cm.	Liver.	Not atypical.	Typical. Rosettes in early stage.	None.	Malignant neuroblastoma.	Photomicrographs.
(19)	1913......	Herxheimer.	M.	1½ months.	Right adrenal.	3 x 3 x 3 cm.	Liver.	Typical.	Typical.	None.	Neuroblastoma sympathicum.	Drawings.
(20)	1913......	Martius.	M.	2½ years.	Right cervical sympathetic.	?	None.	Typical.	Typical.	See remarks.	Malignant sympathoblastoma.	Photomicrographs.
(21)	1913......	Anitschkow.	F.	4 months.	Left abdominal sympathetic.	Fist.	Invaded spinal canal, extradural.	Typical.	Typical.	Mixed.	Malignant neuroblastoma.	Drawings.
(22)	1914......	Wahl.	F.	2½ years.	Left adrenal.	6.8 x 4.4 x 4 cm.	Liver.	Typical.	Typical.	Mixed.	Malignant neuroblastoma.	Photomicrographs.
(23)	1915......	Dunn.	M.	14 years.	Right adrenal.	15 x 10 x 7 cm.	Liver, regional and cervical nodes.	Typical.	Typical.	None.	Neuroblastoma.	Photomicrographs.
(24)	1915......	Glomset.	M.	2 years.	Right adrenal.	9 cm. diam.	Liver, lymph nodes, ribs, and long bones.	Typical.	Typical.	Larger nuclei.	Malignant sympathicus tumor.	Photomicrographs.
(25)	1917......	Lehman.	M.	11 months.	Right adrenal.	13 x 11 x 8 cm.	None.	Typical.	Typical.	Larger cells found.	Neuroblastoma.	Photomicrographs.

Possibly one should add to this list Monro and Dunn's [46] case elsewhere mentioned, and one case of Robertson.[49] However, the tumors in these instances were primarily ganglioneuromata.

Doubtful Cases.

The doubtful cases are divided into two classes:

First: Those that have been diagnosed in the original report as neuroblastoma; second: those that have many of the characteristics of neuroblastoma, but that have been considered sarcomata or other neoplasms.

In the first class belong Wright's cases 2 and 3 (1910),[28] and Symmer's case (1913).[42] The latter tumor occurred in the scapular region of a man of 44 years, after thoracic amputation for a spindle-celled sarcoma of the same site. The histological description of the tumor is characteristic, but the single photomicrograph published is evidence neither for nor against the diagnosis. On account of the bizarre history, it seems wise to class this case as doubtful until more conclusive evidence is presented.

In the second class are placed cases reported by: Parker (1880),[2] Gade (1886, cited by Herxheimer),[4] de Ruyter (1890),[5] Sopetoff (1896, cited by Shukowsky and by Herxheimer — 2 cases),[7] Pitt (1898),[8] Heaton (1898),[9] Pepper (1901),[12] Brück (1905),[15] Küster (1905, this is the second case in Küster's important paper; it is probably a ganglioneuroma).[17] Richards (1905, Cases 35 and 68 of his large collection; Case 65 occurs in the list of undoubted cases),[16] Shukowsky (1909),[26] Glynn (1911–1912).[34] To these must be added numerous cases of malignant tumors of adrenal and liver conforming somewhat to the clinical picture of neuroblastoma, *e.g.*, Ramsay (1899, a study of sixty-seven cases of primary malignancy in the adrenal),[10] Hutchinson (1907, ten cases of primary sarcoma),[22] Frew (1911, "A study of carcinoma originating in the adrenal gland in children").[29] Other such studies have been reported, but they are aside from the purpose.

I have been able to study the originals of all reports mentioned with the exception of those by Gade and Sopetoff. Another source to which I have not had access is a dissertation by Orth (1914); whether or not this paper reports an instance of neuroblastoma, I am unable to say.

[I wish here to express my gratitude to Drs. Harvey Cushing and E. W. Goodpasture for the opportunity to study a related tumor of the central nervous system; to Dr. S. B. Wolbach for a similar opportunity to study the tissue of three of his cases; to Dr. E. A. Baumgartner for access to the embryological collection of the Department of Anatomy of this school; to Dr. G. Swindle for aid in the Bielschowsky technic; to Dr. Opie and members of the surgical staff for valuable advice and unstinted kindness. The photomicrographs were taken by Mr. E. H. Terrill.]

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DESCRIPTION OF PLATES XXV.–XXVIII.

Plate XXV., Fig. 1. — Neuroblastoma of adrenal. Note encapsulation and indentation of surface.

Fig. 2. — Cut surface. Showing hemorrhagic areas.

Fig. 3. — Very cellular sarcoma-like area. Note sparse intercellular substance. C: Strand of connective tissue outlining alveolar structure.

Fig. 4. — Cellular zone and zone almost cell-free, to show transition and alveolar structure. R: Rosette-like body.

Plate XXVI., Fig. 5. — To show two types of cells. E: Immature ganglion cells. C: Stroma.

Fig. 6. — To show two chief types of cell and multinucleated cell. Note shrinkage of parenchyma from stroma. M: Multinucleated cell. C: Stroma.

Fig. 7. — Earliest rosette form. R: Cell groups of six to twelve nuclei. R′ has a cell-free center.

Fig. 8. — Mature rosettes. Note greater density of fibril mass within the cell ring.

Plate XXVII., Fig. 9. — Transitional rosette. The cell ring has begun to separate into two cell clumps with the fibrils disposed between.

Fig. 10. — Transitional rosette, showing a later phase of the process begun in Fig. 9.

Fig. 11. — Most differentiated form of rosette to occur in this tumor. Later stage than Fig. 10. F: Parallel fibrils. A: Cells grouped at ends of fibrils.

Fig. 12. — Cells distributed in parallel chains.

Plate XXVIII., Fig. 13. — To show extensive hemorrhage (black) into tumor tissue and stroma. Alveolar structure clearly indicated.

Fig. 14. — Low power to show situation of atrophied cortex of adrenal. T: Tumor proper. C: Connective tissue of capsule. E: Band of cortical epithelium.

Fig. 15. — High power of adrenal cortical cells shown in Fig. 14.

Fig. 16. — From the adrenal of a 17-millimeter (6–7 weeks) human embryo (Department of Anatomy). To show similarity in cells, fibrillar substance, and cell-grouping.

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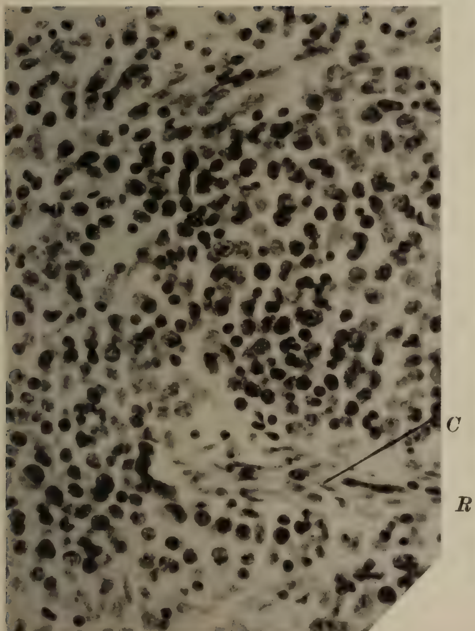

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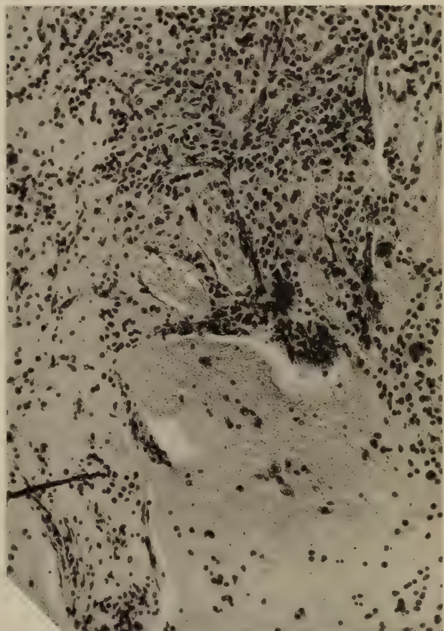

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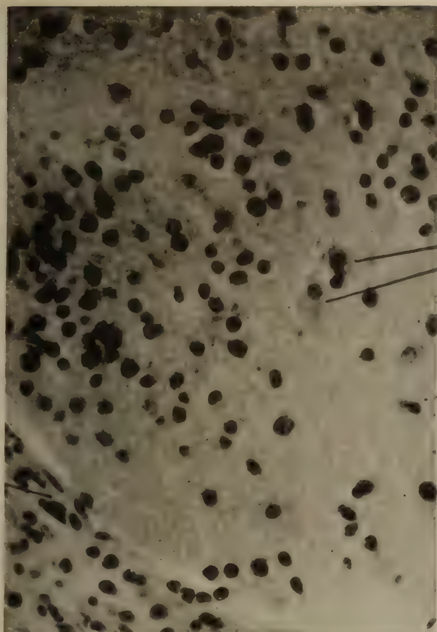

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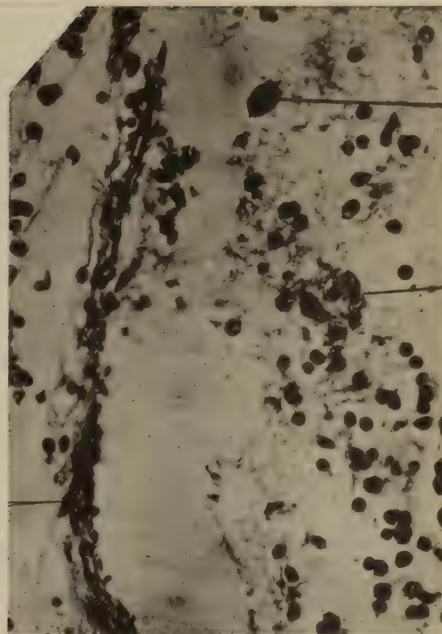

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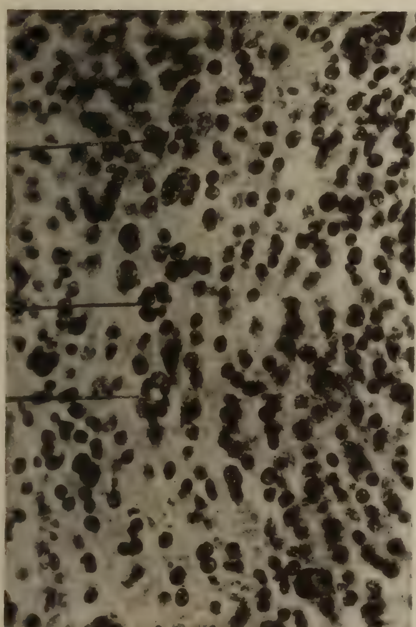

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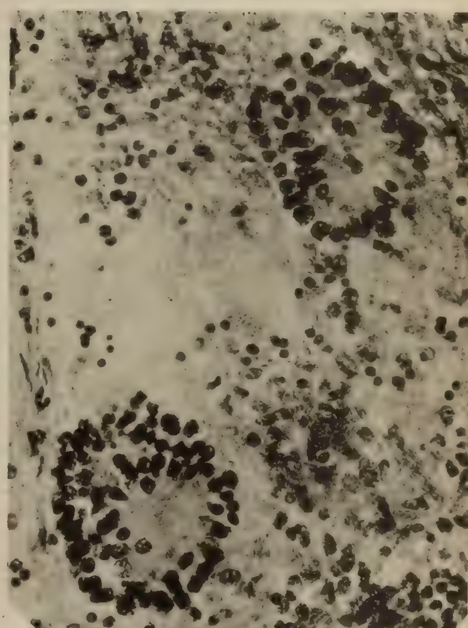

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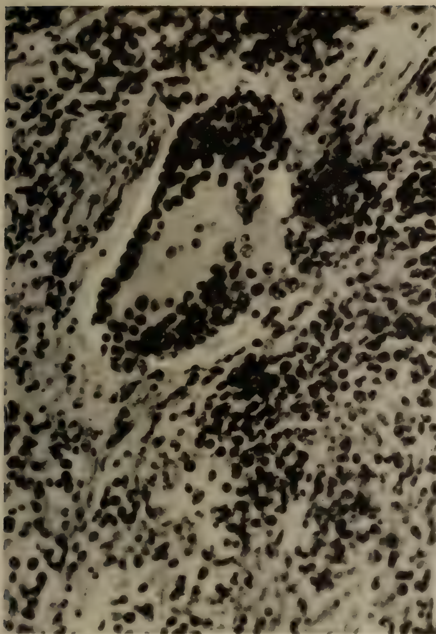

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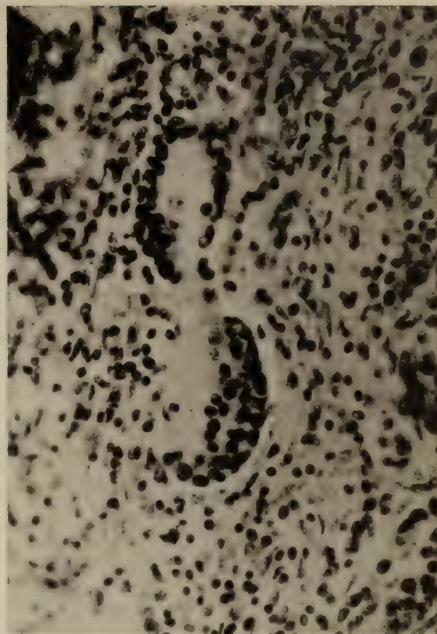

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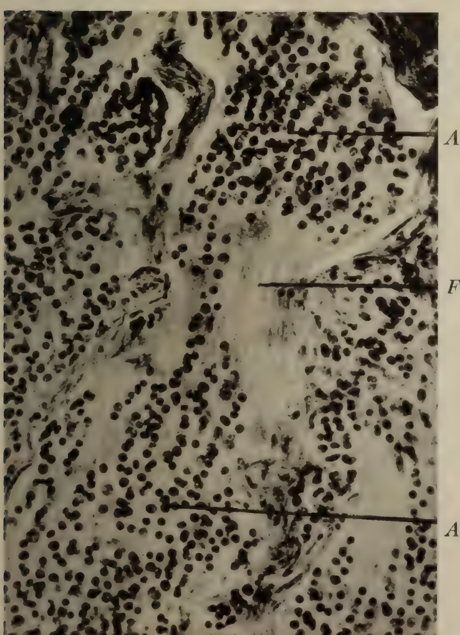

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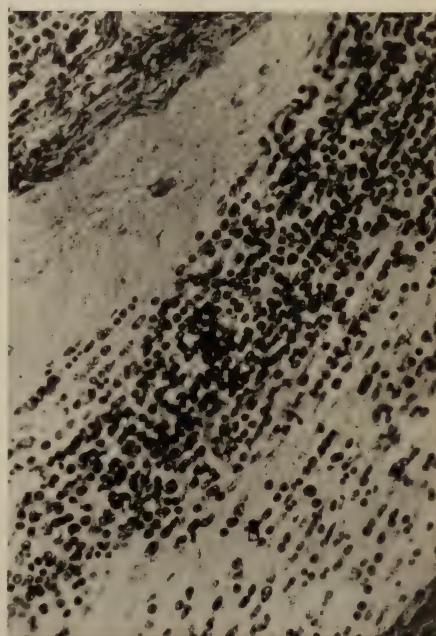

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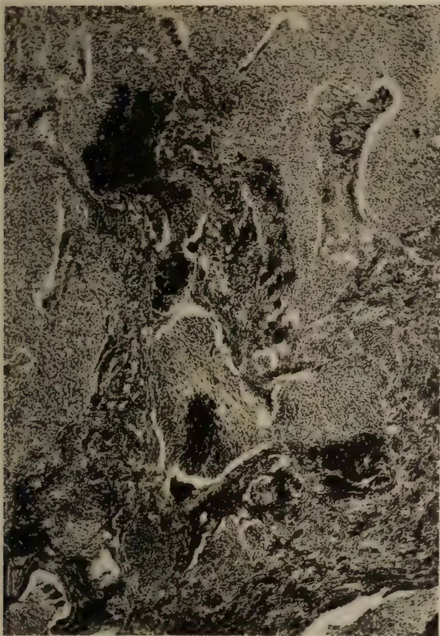

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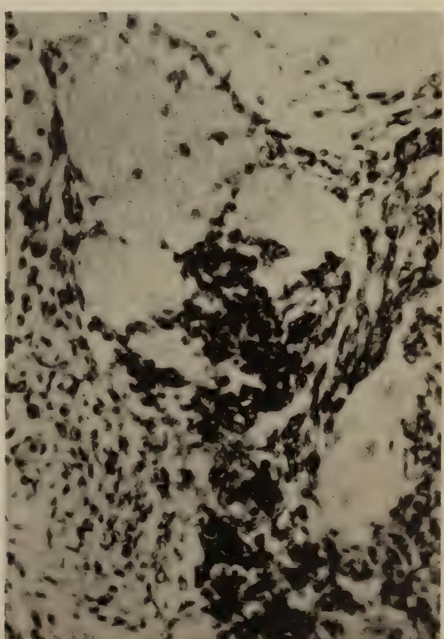

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STUDIES ON THE CIRCULATION OF THE KIDNEY IN RELATION TO ARCHITECTURE AND FUNCTION OF THE ORGAN IN HEALTH AND DISEASE.*

(*First Communication.*)

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The importance of the circulatory arrangement in an organ in relation to its construction and function is evident and well recognized. Knowledge of it furnishes to a great extent the key to knowledge of function. This is nowhere better illustrated than in the kidney.

In normal anatomy and physiology the reconstruction of the circulation in an organ has, therefore, been in the past an important and well-recognized part of anatomical investigation.

Pathologists have been much slower to appreciate its value as regards pathological organ reconstruction. Indeed, until quite recently, pathological anatomists and histologists were quite satisfied to study tissue changes only. Much has, of course, been accomplished in this way, but the simple observations of tissue changes do not disclose the arrangement or architecture of the pathological organ. The importance of the architecture to function, however, is as great in the pathological as in the physiological organ, for many of the pathological functions depend not only upon new tissue elements, but also upon new arrangement of the parts. The importance of this in the kidney was

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fully emphasized by Oertel[1] in 1910. He there sums up his conception of the relation of the total reconstruction of a pathological organ to its function, thus:

> "Here is a future field for pathological anatomy. It can no longer be satisfied to describe and disclose tissue changes, but it *must construe the plan* of the whole pathological organ. Only a knowledge of both will ultimately lead to an intelligent understanding of pathological functions of a whole organ and establish a pathological physiology commensurate with the normal."

The following studies were undertaken to reconstruct the plan of the pathological organs in those chronic kidney diseases which in their functional and tissue alterations offer the greatest and most perplexing changes from the normal. It was deemed advisable to commence with the circulation as the most important, and, at the same time, most promising field for reconstruction.

To this end it was necessary to become first thoroughly familiar with the technic of demonstrating the arterial tree in the kidney.

The old and well-known injections with colored gelatine were tried and gradually a method developed which is detailed below as the first communication to the subject, and principally because it gave results different from the older and clumsier anatomical methods of vascular reconstruction.

Quite recently Ghoreyeb[2] has, by means of an improved injection (Wood's metal), with subsequent corrosion, obtained casts of kidneys which demonstrate much better than before the circulatory conditions up to the last branching of the arteries in normal kidneys. But the metal does not, even in the normal kidney, pass into the glomeruli, whereas the heaviness of the metal as well as the necessary later corrosion seems to introduce sources of difficulties and error, particularly for the study of changes in finer and very tortuous vessels, such as one finds in far advanced cirrhotic kidneys. Furthermore, the organ must subsequently be sectioned in order to obtain anything like a comprehensive conception of the arrangement of the arterial tree. The difficulty of this without disturbing the metal cast is apparent

when we consider the finer changes under pathological conditions.

These possible disadvantages we believe we have overcome and obtained clear and clean injections, including the glomeruli. Beyond these we have, at present at least, not attempted to go.

Our method allows a very clear-cut and plastic (stereoscopic) view of the whole circulation in the intact kidney (Fig. 8), and is accomplished with much greater ease than injection with Wood's metal. The danger of artefacts is therefore reduced to a minimum, practically eliminated.

The apparatus with which this investigation was carried on has proven thoroughly satisfactory and is illustrated in Text Figure I. Without it uniform injection without artefacts is, at least in our hands, almost impossible.

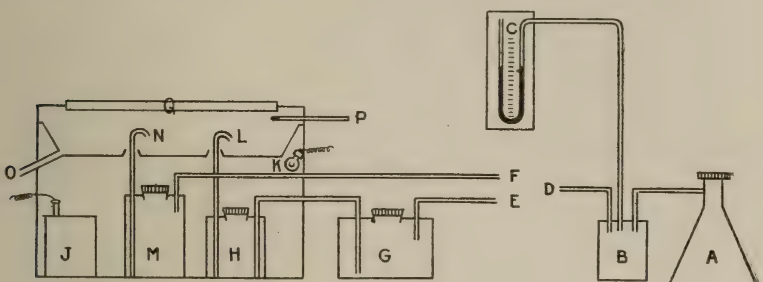

FIG. I.

"A," Compressed air tank which blows air into "B," chamber which registers the air pressure by means of manometer "C."

"D," Pressure tube which can be attached to either "E," inlet tube of saline bottle, or "F," inlet tube of injection mass bottle.

By this means saline can be forced out of bottle "G," into bottle "H," inside the incubator, which is being heated by the immersible electric heater "J," and illuminated by lamp "K."

The saline is thus forced out of bottle "H," through tube "L," into the canulæ which are tied into the kidney vessels.

Similarly by applying pressure tube "D," to "F," the injection mass can be forced out of bottle "M," through "N," into the canulæ of the kidney.

"O," is out-flow tube.

"P," is a thermometer.

"Q," is a glass window for observing the condition of the kidney.

The kidney with the canula tied into the renal artery was washed with normal saline at a temperature of between 45° to 50° C., and at a constant pressure of two hundred millimeters of mercury, until the washings were absolutely clear. This usually took about ten liters of saline, and if the kidney had been obtained soon after death the washings usually left it white. During the washing a slow injection of about two hundred cubic centimeters of a ten per cent solution of potassium sulpho-cyanide is advisable in order to remove rigor of the arteries. (It is, of course, desirable to use fresh organs.)

To begin with, gelatine, colored with either Prussian blue or carmine (the latter was found to be preferable), was now injected at the temperature and pressure stated above. When the whole cortex turned a uniform red, or blue, according to the dye used, all the vessels at the hilus were tied off, the kidney was washed in cold water and put into cold ten per cent formalin.

After forty-eight hours the kidney is usually ready for sectioning, and if it be now cut in half, the cut being made from the convex border of the kidney to the hilus, some very beautiful and instructive pictures can be obtained.

Figure 1 shows a normal kidney so injected. One observes the complete injection of the cortex, and, in striking contrast to it, the pale uninjected pyramids. It was thought at first that our inability to inject the pyramids was due to either the employment of too low a pressure, or that our injection mass was too viscid. But it was found that if pressure was increased rupture of our cortical capillaries resulted — also, that, even if our injection mass was made less viscid, the pyramids would still remain uninjected.

An attempt was now made to inject a small contracted kidney of the chronic productive cirrhotic variety. This was accomplished fairly easily, and upon sectioning the kidney, a striking picture presented itself.

The cortex of this kidney was represented by a very narrow yellowish zone (Fig. 2) in which a few red dots here and there represented the only glomeruli left. Striking,

however, as compared to the normal, was the remarkable even and thorough injection of the pyramids.

The question therefore arose, what was responsible for the lack of injection of the medullary vessels in the normal kidney and the lack of cortical injection, but extremely good medullary injection of the contracted kidney? It is clear that in the normal kidney the injection mass does not reach the medullary vessels — while in the contracted kidney the medullary vessels are easily and completely reached.

It might be suggested that in the contracted fibrous kidney the injection mass cannot pass through the obliterated vessels in the cicatrized cortex; the pressure, therefore, is expended in forcing the fluid into the vessels of the pyramids — in other words, that there is no anatomical change in the arrangement of the vessels in the cortex, but that simply a mechanical alteration of the direction of the pressure determines the injection of the pyramids in the contracted kidney, as opposed to the lack of injection of those of the normal organ.

In order to determine whether this actually was the case, or whether, on the other hand, there really exists a difference in course of the vessels to the pyramids in contracted kidney, we carried out the following experiment:

A fresh kidney was kept in ten per cent formalin until the cortex only had been thoroughly fixed and hardened. This kidney, which now somewhat resembled mechanically the contracted kidney in that the cortical vessels were more or less impermeable, was subjected to injection under exactly the same conditions as the latter. The results were quite suggestive. Portions of the columns of Bertini only, which had not been thoroughly fixed, showed a diffuse injection which consisted mostly of ruptured capillaries — the pyramids, however, showed no injection.

Thus we concluded that the reason for the injection of the pyramids in the contracted kidney was partly obliteration and loss of cortical vessels, partly a different anatomical course to, and arrangement of the blood vessels in, the medulla. It undoubtedly indicates a more open and direct

blood supply for the pyramids in this condition than in the normal kidney.

This had been previously noted, especially, by Oertel,[3] who emphasized that particularly in this form of nephritis a total reconstruction of the vascular architecture occurs, a "short circuit," and he with Ziegler and E. Kaufmann attributes the dilated pyramidal vessels to the elimination of the cortical circulation. Clearly this is an important point in relation to the function of contracted kidneys, for it is evident that secretory alterations must occur as the result of these vascular reconstructions which bring much of the blood into almost direct contact with the medulla and tubular parts contained in it.

In order to examine the vasculature in greater detail frozen sections were made and studied unstained. Figure 3 shows a typical section of the cortex.

While passing some portions of the kidney through the paraffin process for sectioning it was noticed that after thorough dehydration, immersion in xylol which rendered the parenchyma more or less transparent showed up the injected vessels in sharp detail by contrast.

This led us on to prosecute a method which would allow demonstration of blood vessels in larger sections of kidney by rendering the parenchyma transparent. But the xylol method, although showing up thin sections nicely, was useless for thicker ones; moreover, sections soon became opaque.

Aniline oil was next tried with exceptionally pleasing results. Sections even five millimeters thick could be rendered transparent, the blood vessels showing up in clear outline. By using thinner sections one could very easily study the architecture of the interlobar arteries and of the glomeruli, the latter hanging from the interlobars in regular rows of grape-like globules.

The method in detail shaped itself as follows:

(a.) Cut section into strips, preferably 2 to 3 millimeters in thickness.
(b.) Dehydrate in 95 per cent alcohol for 48 hours.
(c.) Dehydrate in absolute alcohol for 48 hours.
(d.) Place in aniline oil.

These sections keep in aniline oil or in glycerine.

However, it was soon found that large and complete reconstruction of the kidney is not possible by this clearing method. We therefore, taking advantage of a suggestion by Dr. Archibald, endeavored to prepare an injection mass such as would show by skiagraphs. It was hoped in this way to obtain a reliable picture of the whole kidney circulation without the tedious and not always strictly accurate method of serial reconstruction.

After trying out several salts we have up to the present been able to obtain the best results by the following injection mass:

Soak 100 grams of gelatine in 500 cubic centimeters of distilled water for 2 hours.

Warm until gelatine dissolves — filter.

To this filtrate add 500 cubic centimeters of a heavy suspension of very fine Barium sulphate such as is used for Barium meals. (Suspension should be as thick as heavy cream.)

Add a crystal of thymol. Stir well and let cool.

With this mass injections were made with results such as shown in Figures 4a, 4b, 5a, 5b.

(The use of skiagraphs in the reconstruction of the circulation in the kidney was employed by Katzenstein (Berliner Klin. Wochenschrift, 1911, No. 36) and later by E. Liek (Archiv. f. Klin. Chir., 1915, cvi, 3) in their experimental studies on collateral circulation in the kidney. They employed metallic mercury, as well as bismuth gelatine suspensions, which were directly injected into the arcus aortæ.

Sampson (Surg. Gyn. and Obstet., xiv, 1912) also used a bismuth subnitrate suspension in gelatine and obtained very good results in reconstruction of the circulation of the uterus. Barium, however, besides being very much cheaper, comes in finer crystals, hence makes a less viscid suspension such as can pass through the very delicate and tortuous vessels in cirrhotic organs. Our apparatus too, does away with most of the difficulties of gelatine injections.

Beck (Surg. Gyn. and Obstet., xii, 1911) shows a beautiful injection of a foot with a bismuth subnitrate suspension in vaseline. Here the additional objections to the bismuth are that injection of the paste must be carried on by means of a syringe, hence accurate estimate of temperature and pressure such as is necessary in delicate injections cannot be ascertained. Section of the organ destroys the injection and it is difficult to preserve permanently even the intact organ. As to the difficulty of this

technic Beck writes on page 7, "the technic in producing perfect results in these anatomical studies requires considerable experience and many failures must precede the attainment of perfect results."

By our method excellent results can be obtained from the first injection.)

Skiagraphs were taken both plain and stereoscopically, and, as is quite evident at a glance, an admirable demonstration of the architecture of the blood vessels (the whole circulatory tree) is thus afforded.

Results obtained in this way indicate that this last method of demonstrating the circulation is applicable to most organs, and that it furnishes a more reliable, rapid, complete, and truthful picture than can be obtained by other methods so far in use.

Certainly some of our findings indicate that, as far as the kidney is concerned, the older conceptions which are still found in anatomical and histological text-books are not borne out by our direct observations of the circulation by means of the skiagraph.

As is well known the conception of the architecture of the renal blood vessels up to the present day dates back to the work of Bowman, Henle, and Ludwig. The renal artery is generally described as breaking up into large branches which arch over the pyramids between the latter and the cortex, and there anastamose. From the arches of these so-called arcuate arteries the interlobar arteries are said to take origin. From these small branches lead into the glomerular tufts. Furthermore, arteriæ rectæ were supposed by Virchow and others to descend from the arcuates and supply the pyramids.

One glance at the skiagraphs will show that there are no divisions of the renal artery which could possibly correspond to these so-called arcuate arteries, and that, instead, the renal arterial architecture resolves itself into a simple tree-like dichotomous arrangement of the branches of the principal afferent artery. These, it is true, run between the pyramids and cortical substance; also they break up rather acutely into their branches, undoubtedly by this means transmitting directly the high aortic pressure.

A remarkable point is the very abrupt and complete breaking up of the large arteries, as they approach the cortex, into numerous very small straight branches which permeate the latter. This abrupt breaking up of comparatively large arteries into very small ones (endarteries) undoubtedly gives rise again to a relatively tremendously high pressure in the latter.

Huber[4] has already shown that there are no arteriæ rectæ which supply the pyramids, and indeed our failure to demonstrate them is in keeping with his findings.

In view of the totally different conception which these reconstructions give of the architecture of the renal blood vasculature, it is perhaps not out of place to suggest a somewhat different terminology for the old one: the first large branches of the renal artery which are seen to course between the pyramidal and cortical substance we would call interlobar arteries — the very numerous small endarteries which run perpendicularly throughout the cortex we would call intralobular arteries — and the small branches which come off at right angles to the latter and run to the glomeruli we would call glomerular arteries.

Figures 6a, 6b, show an arterio-sclerotic kidney injected by this method. One notes the diminution in the size of the cortex and of the vasculature in general, also the withered, gnarled-oak appearance of the vessels. One pole of the kidney shows an infarcted area.

Figure 7 shows a portion of a spleen so injected. It demonstrates the applicability of the process to other organs.

As this communication refers more especially to methods and physiological conditions, a detailed discussion of pathological variations will be taken up in subsequent communications.

[Finally, I wish to thank Dr. Horst Oertel for his invaluable aid throughout this work; also Dr. Cheney and Mr. McNeill for their excellent services in the X-ray Department. To Mr. H. E. Webster, superintendent of the Royal Victoria Hospital, I am also much indebted for his aid in elaboration of technical appliances.]

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EXPLANATION OF PLATES XXIX.-XXXIII.

PLATE XXIX., FIG. 1. — Normal carmine-jelly injected kidney, sectioned.

FIG. 2. — Contracted carmine-jelly injected kidney, sectioned.

FIG. 3. — Frozen section of cortex of normal carmine-jelly injected kidney.

PLATE XXX., FIG. 4a. — Normal barium-sulphate-jelly injected kidney, sectioned.

FIG. 4b. — Normal barium-sulphate-jelly injected kidney, intact.

PLATE XXXI., FIG. 5a. — Normal barium-sulphate-jelly injected kidney, sectioned.

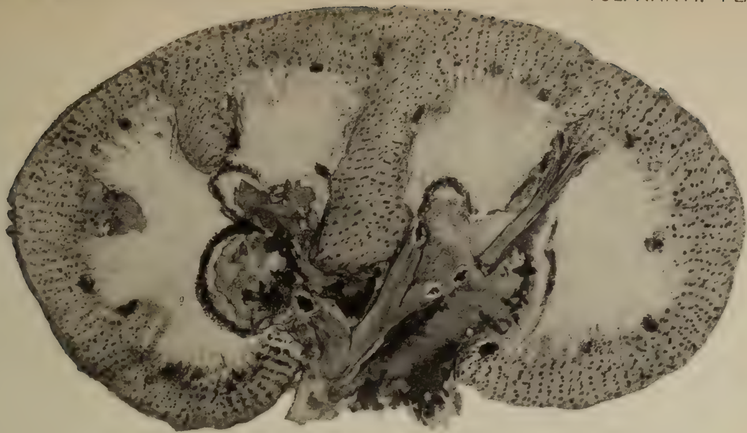
FIG. 5b. — Normal barium-sulphate-jelly injected kidney, intact.

PLATE XXXII., FIG. 6a. — Arterio-sclerotic barium-sulphate-jelly injected kidney, sectioned.

FIG. 6b. — Arterio-sclerotic barium-sulphate-jelly injected kidney, intact.

PLATE XXXIII., FIG. 7. — Barium-sulphate jelly injected spleen.

FIG. 8. — Stereoscopic view of arterio-sclerotic kidney sectioned. This plate may be cut out, mounted on a cardboard, and viewed through the hand stereoscope.



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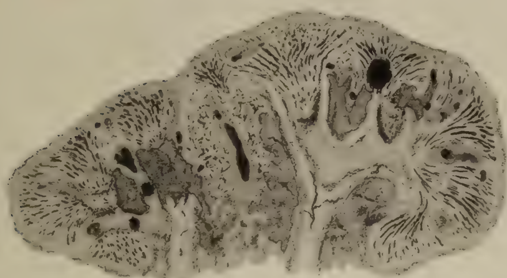


2

3

Gross.

Kidney.

4a

4b

Gross.

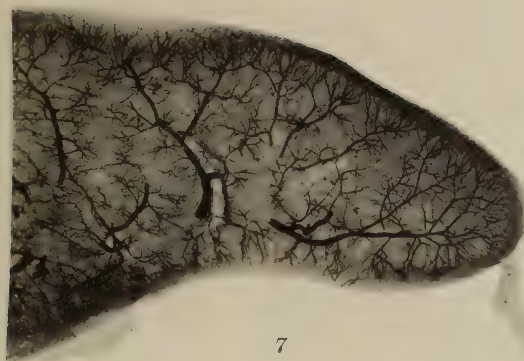
Kidney.

5a

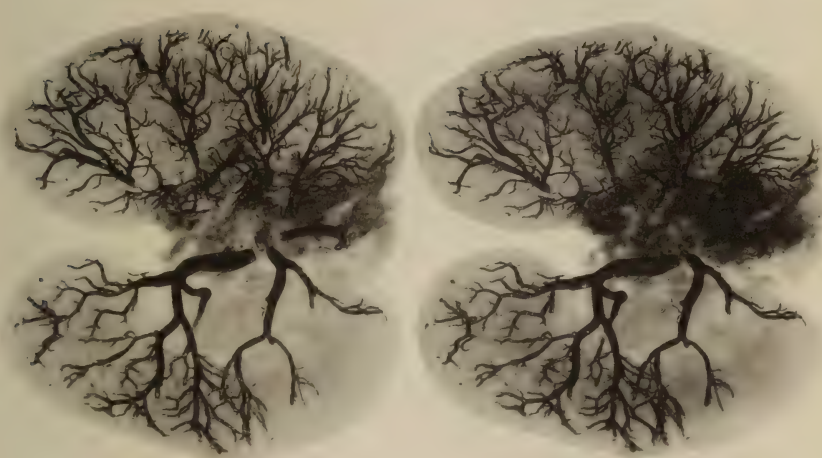
5b

6a

6b



7



8

A NEW PATHOGENIC SPOROTRICHUM.

FOUND IN A CASE OF ACUTE ARTHRITIS OF THE KNEE FOLLOWING INJURY (SPOROTRICHUM COUNCILMANI).*

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Instances of infection with sporotricha following injury, in America, are of unusual interest in the consideration of the distribution of presumably free living pathogenic fungi. The study of the culture from the case here presented has revealed a new variety of sporotrichum, for which we propose the name Sporotrichum councilmani.

The clinical aspects of the case were unusual and probably peculiar to the nature of the infecting organism, and for that reason a complete clinical history is given.

Clinical history. — The patient (Hosp. No. 3414), a boy of ten, entered the Peter Bent Brigham Hospital, Boston, Massachusetts, complaining of pain in his right knee. One week before admission, while playing in an ash barrel, his right knee was punctured by a nail. Two days later the knee-joint became slightly swollen and painful. His normal activities, however, were not interrupted until twelve days later, when he came to the hospital because of severe pain in his knee. The family and past history of the boy were entirely unimportant. The physical examination on admission showed negative findings except for marked swelling of the right knee-joint. The leg was held in a flexed position, apparently due to the existing muscular spasm. There was little redness of the affected part and but slight rise in local temperature. There were two small punctate abrasions over the inner aspect of the joint caused by the nail injury. Palpation and all movements of the joint elicited excruciating pain. An examination made under general anesthesia revealed no crepitus or restriction of movements of the joint. A sense of fluctuation was easily made out. The patient's temperature on admission was 99° F. During his stay in the hospital the temperature was intermittent in character, frequently reaching a maximum of 102° F. The pulse rate remained about 100 per minute.

* Received for publication April 5, 1917.

The joint was aspirated repeatedly without relief. Large amounts, 50–60 cubic centimeters of fluid, were recovered. Other treatment consisted of introducing a sterile solution of gentian violet into the joint cavity and irrigation with a bichloride of mercury solution. Large doses of iodine were given by mouth. None of these therapeutic measures gave relief. The condition was unimproved after two months. The patient's general health remained excellent in contrast to the acuteness of the local condition. After two months the joint became immobilized. The swelling and tenderness gradually disappeared and the patient was discharged five months after admission, free from any symptoms of an acute arthritis, but with complete fixation of the right knee-joint.

Clinical pathological findings. — White blood counts, made repeatedly, ranged from 9 to 14,000. The differential count showed 77 per cent polymorphonuclear cells, 7 per cent basophiles, 5 per cent large mononuclear cells, 9 per cent small mononuclears, 2 per cent eosinophiles. The von Pirquet cutaneous skin tuberculin test was negative, as was the Wassermann reaction for syphilis. Repeated X-ray examinations showed no bony involvement. Fifty cubic centimeters of light brown peculiarly mucoid fluid was aspirated one day after admission. Microscopical examination of the fluid showed numerous pus cells.

Cultures made on plain agar showed a profuse fungus-like growth. The medium was thought to be contaminated because of the appearance of this growth and cultures were therefore discarded. The characteristics of the fluid recovered from various aspirations in each instance were similar to those described above. Suspicion was aroused by the repeated presence of the fungus-like growth from the aspirated fluid and careful study of the growth was then undertaken.

Serological studies. — Soon after the isolation of the fungus, complement fixation and agglutination tests were made. For both of these tests suspensions of the spores from potato cultures in normal saline solution were used. The spores were freed from particles of the medium and from hyphæ by filtration through filter paper. Unfortunately no record has been kept of the number of spores per volume in the suspensions used as antigen for the complement fixation tests; Widal and Abrami (Ref. 1, Les Sporotrichoses, p. 574) used simply an opaque suspension of the spores; —

the character of the medium and age of culture they state is immaterial. The tests were made by Dr. I. C. Walker, in charge of the Wassermann Laboratory at the Peter Bent Brigham Hospital.

COMPLEMENT FIXATION TESTS.

Patient's Serum.	Antigen.	Complement.	Salt Solution.	Sensitized Cells.	Result.
0.1 cc.	0.1 cc.	0.5 cc.	0.8 cc.	1 cc.	++
0.2 "	0.1 "	0.5 "	0.8 "	1 "	++
0.1 "	0.2 "	0.5 "	0.8 "	1 "	++
0.1 "	0.5 "	0.5 "	0.4 "	1 "	++
0.2 "	0.5 "	0.5 "	0.4 "	1 "	++
0.1 "	0.5 "	0.5 "	0.4 "	1 "	++
	Diluted 1–10 cc.				
0.2 "	0.5 "	0.5 "	0.4 "	1 "	++

These tests were controlled by substituting for the patient's serum (1) serum from a proved syphilitic, and (2) serum from a proved normal individual. In both instances there was no fixation of complement.

AGGLUTINATION TESTS.

Dilutions.	Patient's Serum.	Control.
1–25	+	O
1–50	+	O
1–100	Slight Agglutination.	O
1–200	O	O
1–300	O	O

On the repeated isolation of the culture from fluid aspirated from the joint and on the positive complement fixation and agglutination tests we base our conclusion that the sporotrichum was the responsible infectious agent in this case.

Pathogenicity. — The cultures used for inoculation were usually grown upon potato. Fresh cultures, containing growing hyphæ as well as spores, were always used. The cultures were ground with a heavy spatula-shaped platinum needle against the inner side of a test-tube, and suspended in normal salt solution. The injection material therefore consisted always of fragments of hyphæ and spores.

White mice, white rats, guinea-pigs, and rabbits were used for the study of pathogenicity. In all these animals intraperitoneal inoculations produced discrete nodular lesions, usually upon the omentum, but occasionally between coils of intestines and upon the surfaces of the liver and spleen. Localized injections subcutaneously and into the knee-joints of rabbits and guinea-pigs produced merely local inflammatory reactions, granulomatous in character and histologically similar to lesions occurring after intraperitoneal injections. Intravenous injections into the tail veins of rats, which proved to be the most susceptible animal, produced fatal results following striking cerebral symptoms with the production of miliary abscesses in the cerebrum, cerebellum, and various organs.

The lesions following injections into the tissues and intraperitoneally were always purely local. Metastasis to other parts of the body did not occur. It was always possible to demonstrate spores in the lesions and to obtain pure cultures of the organism up to ten weeks after inoculation, which was the time of the longest experiment.

The histology of the lesions is simple and resembles that produced by other pathogenic fungi, particularly the actinomyces and the organisms of cutaneous blastomycosis and coccidioidal granuloma. The first reaction is the collection of polymorphonuclear leucocytes and endothelial (epithelioid) cells about the organisms. Giant cell formation and

the taking up of the spores by endothelial cells and giant cells occurs. The mycelial fragments always seem to disappear soon after injection. At the periphery of the lesions fibroblast proliferation becomes active. Early lesions therefore consist of a central mass of polymorphonuclear leucocytes, endothelial cells, and giant cells surrounded by a zone of fibroblasts. The lesion progresses only following germination of the spores with the production of branching filaments radially arranged. The filaments may extend into the zone of fibroblasts, causing the production of other small abscess-like collections of polymorphonuclear leucocytes. Late lesions, of three to four weeks' duration, consist of fibrous nodules containing several foci of soft yellowish caseous or puriform material.

Illustrative cases. — Guinea-pig 2, Series III., killed four weeks after intraperitoneal inoculation with a suspension of a potato culture.

Autopsy findings. — There is a firm mass about 1 centimeter in diameter at the site of injection. The surrounding tissues are indurated. Three loops of intestines are attached to the peritoneum underlying the omentum. The mass consists chiefly of yellow, cheesy material encapsulated by a fibrous wall. The omentum contains a number of tubercles varying from 2 to 8 millimeters in diameter. These, on section, show characteristics similar to the mass in the abdominal wall. Some of the tubercle-like formations are adherent to the pancreas, others to the liver and to loops of intestine. A node about 8 millimeters in diameter is found in the region of the celiac axis. This also contains yellow necrotic material. The mediastinal, retroperitoneal, and pelvic lymph nodes are definitely enlarged, but on section show no focal lesions. Isolated small yellow areas are found at the hilus of the liver, at the lower pole of the spleen, and in the upper lobe of the left lung. Preparations made from these tubercle-like lesions show spores. Cultures made from the necrotic material also show a growth typical for the fungus injected.

Rabbit 1, Series IV. One cubic centimeter of a suspension of spores of the fungus was injected into the second joint of the left leg. At the end of two and a half weeks the joint was swollen and the animal was unable to use the left

leg. Manipulation of the joint apparently caused pain. The animal was killed three and a half weeks after injection.

Autopsy findings. — The second joint of left leg is definitely swollen, there is definite fluctuation of the soft parts. One cubic centimeter of slightly sanguinous, thick, mucoid fluid is recovered from the joint. There is slight injection of the synovial membranes. The latter, however, are smooth and glistening. The bony structure of the joints show no gross changes. X-ray examination of this joint likewise shows no bony changes. Smears from the fluid recovered show occasional spores.

The lesions following intravenous injection of suspensions of spores into white rats are acute in character. The animals within seven days usually showed symptoms of cerebral involvement and if not killed would succumb to the infection. At autopsy miliary-sized abscesses are found in the brain and kidneys, and usually an abscess of considerable size in the tissues adjacent to the site of inoculation, *i.e.*, base of tail. Microscopical examination shows minute lesions in the lungs and occasionally lesions in the spleen.

The histology of the lesions following intravenous injection of the spores into white rats shows two types: (a) Lesions due to lodgment of spores with a possible slight proliferation of very short abbreviated hyphæ (gonidial budding?), and (b) those following the development of colonies of the organism in filamentous form. The first type of lesion consists of collections of giant cells and endothelial cells with occasional polymorphonuclear leucocytes, and is situated adjacent to small blood vessels — brain, kidneys, and lungs. The only organisms demonstrable in these lesions are spores and oval forms — possibly short mycelia. The second type of lesion, common in the cerebrum, cerebellum, and kidney, and occasionally in the spleen, are small abscesses and consist almost wholly of polymorphonuclear leucocytes surrounding colonies of the fungus in the form of branching radiating filaments. The filaments are thicker, with shorter segments, than those found in cultures (Figs. 22 and 23).

For demonstration of the filaments in tissues we have found Mallory's connective tissue stain after Zenker's fixation to be the most satisfactory, for the reason that the protoplasm of the filaments is difficult to color with any stain. The connective tissue stain brings out the membrane of the filaments distinctly, blue in color, while the surrounding cells are stained red.

Illustrative case. — Series V., Rat w 191. Inoculated February 5th with suspensions of spores from a potato culture in normal saline solution, into the tail vein and intraperitoneally. On February 10th the animal was found lying on the floor of the cage. When disturbed it would roll over repeatedly in attempts to gain its feet. When resting its head was sharply drawn to one side and it would frequently convulsively bite its hind paws, which were lacerated and bleeding from this cause.

Autopsy showed miliary-sized abscesses in the cortex and basal ganglia of the cerebrum, in the cortex of the cerebellum, and in the kidneys. At the base of the tail there was found a pocket of thick tenacious puriform material extending into the pelvis, between the rectum and the spine. In the peritoneal cavity there was a thick exudate on the surface of the spleen and liver and in the rolled up omentum.

Description of the fungus. — The organism, which was early identified as a sporotrichum, grows readily on all ordinary culture media at temperature between 20° and 38° C. The optimum temperature is about 30° C.

The gross characteristics of the cultures resemble those of Oidium lactis during the first few days, or before pigment production is noticeable. It exhibits marked differences from the appearances of cultures of other pathogenic sporotricha. Cultures of Sporotrichum schenki and Sporotrichum beurmanni sent by Dr. D. J. Davis of Chicago, cultures designated as 671 and 672 sent by Professor C. E. A. Winslow, then Curator of the Department of Public Health of the American Museum of Natural History, and the cultures described by Page, Frothingham, and Paige [2] from a case of sporotrichosis in a horse, were used for comparison. The

culture of Sporotrichum schenki is the original culture described by Hektoen and Perkins (Jour. Exper. Med., v, 1900–1901, 77), and was given to Doctor Davis by Professor Hektoen. The culture of Sporotrichum beurmanni was given to Doctor Davis by Sabouraud in Paris. Culture 671 was isolated by Professor K. F. Meyer from a case of sporotrichosis in a horse in Pennsylvania in May, 1912, and is probably the culture designated strain "C-10" in his paper, "Various Sporotricha differentiated by the fermentation of carbohydrates."[6] Culture 672 was also isolated by Meyer from a case of accidental human infection of the arm in a laboratory worker, in April, 1913, and probably is the culture designated as strain "CC" in the above paper.

There are several characteristics (permanent over a period of two years) of the sporotrichum isolated by us which are common to all media and which make its identification easy when compared with the other sporotricha studied: (1) The gross cultural appearances are markedly different, our sporotrichum always produces a profuse aerial growth of hyphæ, occasionally completely filling the test-tube with a white cotton-like growth (Fig. 12). This has not been seen with the other sporotricha. (2) Microscopically the hyphæ and spores are distinctly larger. (3) The production of clusters of lateral spores or conidia from vegetative hyphæ, so characteristic of other sporotricha, does not occur.

In common with other sporotricha there is a great variation in the gross appearance of the cultures, both in regard to the form of the growth and in pigment production. A detailed description follows:

Plain agar. — Growth is visible within 24 hours as small, flat, circular colonies, translucent, grayish in color, with fine radial striations. After 36–48 hours the colonies become small isolated white tufts, forming cotton-like masses which may be elevated 5–8 millimeters above the surface of the medium. Occasionally the aerial growth takes the form of radiating grayish white spikelets (Fig. 8) formed by the coalescing of filaments — the "*filaments en agrégé*" of De Beurmann and Gougerot.[1] After a few days the growth in contact with the medium becomes deeply pigmented, brown to black, and the medium becomes colored brownish. After two or three weeks the surface of the white aerial growth becomes covered

with a dry powdery layer of spores which may be white, but which is usually colored; the color on agar is usually a rusty brown, sometimes, however, it is a mouse gray. The latter color is more common on starch containing media, notably potato (Fig 13). When the growth has taken the spikelet form these become covered with plume-like masses of white or brownish spores. The growth penetrates the surface of the agar for a distance of one or two millimeters.

Dextrose agar. — The growth is similar to that on plain agar although more rapid and usually more pigmented (Fig. 11). The coloration of the medium is more marked. Old cultures, three to four weeks, usually show a jet black substratum in contact with the agar, with white and grayish cotton-like hemispherical masses of aerial growth, covered with a rusty brown or grayish spore layer.

Glycerine agar. — The growth resembles that on dextrose agar.

Potato slants. — Growth on this medium is very rapid, and within three or four days the inoculated surface is usually covered with a white, dry, loose textured, cotton-like layer 2 to 5 millimeters deep (Fig 12). Later this growth may completely fill the tube around the medium. Occasionally on potato the spikelet form occurs in the initial colonies, but is later replaced by the white cotton-like growth. Rarely the initial growth on potato is moist and takes the form of a smooth, shiny, white, gray or black layer (Figs. 9 and 10), which finally becomes covered with a powdery layer of spores or a heavy white cottony growth. After one or more weeks the growth in contact with the medium becomes a dark greenish gray or black (Fig. 12), while the surface of the aerial growth becomes covered with a mouse gray layer of spores (Fig. 13). If the cultures are allowed to dry slowly the color of the growth becomes dark brown to black. Completely dried potato cultures, however, always retain a separable layer of filamentous growth in contrast to the other sporotricha, which yielded only dense crusted masses of spores separable with difficulty from the medium.

Bouillon. — The growth in bouillon and in mixtures of bouillon with ascitic fluid occurs as spherical white colonies resembling thistle-down in texture. The surface growth is at first white, but later may become pigmented as on agar media.

Gelatine. — Nutrient broth gelatine is slowly liquefied. The surface growth undergoes the changes exhibited by colonies on agar.

Blood serum. — Loeffler's coagulated blood serum is slowly liquefied. The spikelet form of growth is very common on this medium. Pigmentation is not as marked as on the other media used.

Starch agar. — There is a slight clearing of the medium in immediate contact with the growth.

Nitrate broth. — No reduction of nitrates occurs.

Peptone solution. — Indol is not formed; the reagents used were sulphuric acid and paraldehyde.

Litmus milk. — There is coagulation of the milk, in one to three days, without change of color. The clot produced is soft. The liquid after

separation of the clot remains clear, the color changes to a bluish purple while the clot remains blue. The clot after several days breaks up and is deposited as a blue flocculent sediment. The supernatant liquid remains purplish. After several weeks, if a surface pellicle of growth is allowed to form, the litmus becomes decolorized.

Fermentation tests. — These tests were done with sugar-free broth to which the substance to be tested was added in solutions sterilized at 100° C. for twenty minutes. As the fungus grows rapidly, the tests by titration were done at short intervals; three tests were made after six, ten, and seventeen days' growth. A control tube of each substance tested was kept and titrated at the same intervals.

The following table shows the results obtained. Gas was not produced with any test substance.

	Six Days.		Ten Days.			Seventeen Days.	
	Control.	Culture.	Control.	Culture.	Culture.	Control.	Culture.
Dextrose	.....	1.8	0.4	1.6	.....	0.3	1.1
Saccharose	.....	0.3	0.3	Neutral.	Trace acid.	0.4	Neutral.
Lactose	0.4	0.4	0.3	0.5	Trace acid.	0.4	"
Mannite	0.4	0.6	0.3	0.5	Trace acid.	0.2	Trace acid.
Inulin	.....	0.5	0.5	0.3	0.5	0.5	Neutral.
Glycerine	0.4	0.4	1.2	1.7	2.0	1.8	2.0+
Dulcite	.....	.....	0.5	1.9	1.8	1.5	1.5

The results summarized indicate that dextrose, glycerine, and dulcite are fermented. The reaction with glycerine and dulcite apparently returns towards the starting point after an initial increase in acidity. With the non-fermented sugars, saccharose and lactose, the reaction becomes definitely more alkaline.

The fermentation tests of this sporotrichum agree with the results of Meyer and Aird,[6] for two strains of Sporotrichum schenki and one of Sporotrichum beurmanni. The results of Meyer and Aird, however, are at variance with those of

Blanchetière and Gougerot,[1] who state that S. schenki produces acid in inulin and lactose, as well as in dextrose and glycerine, while S. beurmanni produces no acid with lactose, but does with dextrose, glycerine, saccharose, and inulin. A third sporotrichum, S. gougeroti, has its fermentation tests given by Blanchetière and Gougerot as follows: Positive for dextrose, glycerine, and saccharose, negative for lactose and mannite.

We are unable, because of the discrepancies noted in published data, to obtain information of value in the placing of the sporotrichum isolated by us. It is perhaps possible that Meyer and Aird have made a mistake in not titrating their cultures at shorter intervals, for it seems possible that the reaction with some test substances may return towards the starting point after an initial acidity. The microscopic appearances of the cultures are in general similar to those of other pathogenic sporotricha, except that our organism does not produce the clusters of lateral spores so characteristic of the other sporotricha studied by us. Chlamydospores, or sclerotic cells (Figs. 14 and 17b), occur in old cultures, and particularly in the growth from tissues from experimentally inoculated animals. There is much less tendency for our sporotrichum to grow in yeast-like forms (gonidial budding?) than occurs even in early cultures with the other sporotricha we have studied. Large budding forms characterized by a large number of food granules (reserve cells?) have been observed in cultures grown on animal tissues (Fig. 17a). The dimensions of the spores and hyphæ are larger than those of the other sporotricha studied.

De Beurmann and Gougerot give the following dimensions for the spores of various sporotricha: Sp. schenki, spores $3 \times 5\mu$; Sp. beurmanni, spores 2 to $4\mu \times 5$ to 6μ; Sp. gougeroti, spores 4 to 8μ in length.

Our own measurements yield dimensions for the spores of Sp. schenki, $2\mu \times 3.6\mu$ to $2.7\mu \times 4.2\mu$; the sporotrichum of Page, Frothingham, and Paige, $2.5\mu \times 3.6\mu$ to $2.1\mu \times 4\mu$; for Cultures 671 and 672, $2\mu \times 3.6\mu$ to $2.7\mu \times 4.2\mu$. The dimensions of our sporotrichum are from 2.5μ to $4\mu \times 6\mu$ to

8μ. The diameter of the hyphæ as well averages somewhat larger for our sporotrichum than for the others. The variation, however, with the age of the culture and character of the medium makes this datum of less value. We would, however, fix the diameter of the hyphæ of our culture at 2μ, while the diameters of the hyphæ of the other cultures studied were less than 2μ. The method of spore formation was studied particularly by F. C. Meier, who has contributed the following account:

"The Van Tiegham cell was used in order to study the method of spore production, but after some preliminary experimentation it proved best to employ a 1½-inch stender dish instead of the smaller types of cell. In this way oxygen was supplied to the plant in sufficient quantity with the result that the growth seemed to be more normal than was the case when the smaller receptacle was used. Since, however, such an arrangement, while excellent for observing the germination of the spores and the general nature of the growth, is not well adapted for securing permanent preparations, the following procedure was adopted:

"A 2½-inch stender dish containing a smaller 1-inch vessel was placed within a 6-inch crystallizing dish. Three ½-inch cover-glasses were spread out on the bottom of the small inside stender dish, which was allowed to remain open, the two dishes which enveloped it being capped by covers which overlapped the edges. The space left between the dishes was loosely filled with filter paper to prevent them from sliding about. This combination was then sterilized by dry heat.

"When it was desired to make a preparation, in order to maintain a moist atmosphere within the cell, sterile water was poured in the large stender dish, enough being used to surround the small dish, which contained cover-glasses. This being done a small drop of potato agar was placed on each cover-slip and a spore inoculation was made. After several days' growth, the resultant colony could be killed and stained in situ, simply by running in and pipetting off the fluids used. When the process of staining had been completed, the cover-slips were removed and mounted

"As one observes the life history of the organism when it is growing in the Van Tiegham cell, it is evident that there is one distinctive type of spore formation. An aerial branch, which is to assume the function of sporophore, buds off from the vegetative hypha. In its early stages the basal portion of this branch may be slightly swollen, but if the growth is rather extensive such differentiation gradually disappears. When spore production commences, the sporophore becomes somewhat papillate at the distal end (Figs. 7a and 15). This swelling increases in size, is separated from the branch by a septum, and finally assumes the shape of a piriform spore, which is slowly pushed aside as the sporophore continues

to elongate along the line of its original axis (Fig. 2b). As a result of the continued elongation, a new spore forms which in its turn is pushed aside, usually at a different angle from the first, to make way for the next. Following this scheme of development a compact cluster of caducous spores is ultimately produced at the tip of the sporophore (Figs. 18, 19, 20, and 21). When a spore has been shed a slight denticulation remains to mark the point of attachment (Fig. 6).

"From the following description, quoted from 'Les Sporotrichoses,' it is evident that Matruchot has observed a process similar to this in Sporotrichum schenki:

"'Dans les regions fertiles aériennes, c'est-a-dire dans les parties de la culture où la fructification se fait normalement, les conidies naissent de la façon suivante: sur une branche du mycêlium apparait, d'abord l'extremité, un petit renflement qui devient une spore. Lorsque cette spore est formée, une deuxième apparait à côté ou un peu au-dessous; puis une troisième, et il se fait ainsi un petit bouquet de spores nées isolément et successivement.'

"In addition to this specialized type of spore production, there are secondary methods. In rare cases the development may be of the following nature: A bud arises from the vegetative hypha as though a branch were to be formed. Elongation, however, gives place to enlargement, the swelling is separated from the hypha by a septum and a spore results, which in all respects but that of position agrees with those formed in the normal cluster (Figs. 1 and 16a). In other instances a variation of this method may occur, whereby the bud develops as in the case just described, then grows out at the apex, forming a secondary enlargement which may unquestionably be considered a spore (Figs. 4 and 16b).

"Saccardo, in the 'Sylloge Fungorum,' gives the original diagnosis of the genus Sporotrichum as follows: 'Sporotrichum Linp. Sp. Pl. Fungi, Pl. em. Sacc. Mich. 2, p. 16 (Etym., *spora* et *thrix* pilus). — Hyphæ vage iteratoque ramosæ, septatæ v. continuæ, solito procumbentes aequales. Conidia in ramorum v. denticulorum apicibus acrogena, solito subsolitaria, ovoidea v. subglobosa. —'"

Summary by Doctor Meier: "Hyphæ, 1μ to $1\frac{3}{4}\mu$ in diameter. Branching, irregular, the young sporiferous branch often somewhat swollen in the proximal region; septate, the septa occurring at intervals of from 10μ to 22μ. Spores hyaline, one-celled, piriform, 7μ to 6μ x $2\frac{1}{2}\mu$ to $3\frac{1}{2}\mu$, solitary or usually in clusters formed by successive apical proliferation at the tips of aerial sporophores, rarely sessile on the walls of the vegetative hyphæ, the point of attachment being marked by a slight denticulation of the fertile branch."

No satisfactory basis for the classification of varieties or species of sporotricha has been determined. The botanical criterion for the genus sporotrichum is the manner of spore formation. For the separation into species and varieties

De Beurmann and Gougerot[1] rely upon gross cultural characteristics, fermentation tests and morphological peculiarities in animal tissues as well as in cultures.

The gross cultural characteristics of the pathogenic sporotricha are extremely variable for any one culture and De Beurmann and Gougerot were the first to emphasize the pleomorphic character of the colonies. Under pleomorphism are considered four types of variation:

1. Variation in color; *i.e.*, white, browns, and black.
2. Form of growth; *i.e.*, surface contour, wrinkling, etc.
3. Formation of filaments en agrégés; *i.e.*, prickle-like, plume, and tress-like growths from the surface of cultures.
4. Variations due to surface deposits of spores giving rise to variously colored powdery surface appearances.

Data based on fermentation tests are as yet too meager to be of value. Meyer and Aird[6] conclude that "the reactions are not fixed and are as inconstant as the many variations noted in the formation of chlamydospores, and frequently in pleomorphism." On the other hand, the results obtained by us indicate constant behavior, for our culture, which is the only one we have carefully studied, and Blanchétière and Gougerot[1] have apparently found constant fermentative differences between three species of sporotricha, namely S. schenki, S. beurmanni, and S. gougeroti.

Minor morphological variations have been used by De Beurmann and Gougerot to establish varieties of S. beurmanni.

De Beurmann and Gougerot[1] present (page 47) the following grouping of pathogenic sporotricha:

Group I.

Sporotrichum (ancestral)....	Sporotrichum schenki-beurmanni. Common stock for	Sp. schenki (1). Sp. beurmanni and its varieties (2). Sp. beurmanni variety asteroides (3). Sp. beurmanni variety indicum (4). Sp. jeanselmei (5).

Group II.

Sporotrichum gougeroti (6).
Sporotrichum (?) dori (7).

In the above classification Sporotrichum gougeroti is separated from Group I. because of very (1) constant gross cultural characteristics — uniform black color and absence of pleomorphism; (2) important morphological differences, larger size of filaments and spores, and tendency to short filaments or moniliform growth; (3) differences in fermentation properties, and (4) differences in chemical analysis of the cultures.

Sporotrichum dori is a doubtful member of this genus, according to De Beurmann and Gougerot. The cultures have been lost. The morphology approaches that of Nocardia, while the manner of fructification is undetermined. The reasons for De Beurmann's and Gougerot's consideration of this organism with sporotrichum are obscure.

Sporotrichum beurmanni var. asteroides and Sp. beurmanni var. indicum are poorly defined. The former is distinguished chiefly by the formation of bizarre radial forms in tissues as yet poorly studied, and the latter is retained apparently out of deference to Castellani, as no evidence for separating it from Sp. beurmanni has been presented. The description of both of these cultures is too meager for comparison with the other sporotricha, while no fermentation tests at all are reported.

Microscopically, Sp. schenki and Sp. beurmanni are practically identical in morphology. The greatest differences are in the gross cultural appearances. Sp. schenki produces little or no pigment, while Sp. beurmanni always produces dark-colored growths, chocolate brown to black. There are also differences in the contours of the surface growths; the cultures of Sp. beurmanni present a convoluted surface, irregularly intercrossed like the cerebral convolutions, Sp. schenki presents sharply contoured rectilinear elevations, often radiating from a center like the "valleys about the cone of a volcano." Microscopically, the mycelium of Sp. schenki is often curved or undulating, usually clustered and parallelly arranged, without interlacing or entanglement The mycelium of Sp. beurmanni is straight, sometimes clustered, rarely parallel, and almost always interlacing or entangled. Sp. schenki produces fewer spores. Sp. beurmanni produces many spores. Lateral gonidiophores are common to the latter, rare or absent with the former. Sp. schenki ferments lactose, not saccharose, while Sp. beurmanni ferments saccharose, but not lactose.

Sporotrichum jeanselmei has been incompletely studied; no biological tests have been made. Microscopically the cultures are identical with Sp. beurmanni. The separation of Sp. jeanselmei from Sp. beurmanni apparently rests solely upon gross differences in the cultures, chief among which are the following characteristics for Sp jeanselmei: (1) a powdery, or downy, or hairy surface to the growth, especially upon sugar containing media colored white or mouse gray, even though the growth in contact with the medium has assumed an ink-black color; (2) a great variation in growth (pleomorphism) upon dextrose containing media, not shown by Sp. beurmanni, and (3) the profuse growth yielded upon

simple media (without addition of sugar) as compared with the poorer growth of Sp. beurmanni.

De Beurmann and Gougerot state that some appearances of Sp. jeanselmei are identical with certain pleomorphisms of Sp. beurmanni, yet the two cultures can always be identified upon study.

From the above summary of the classification and reasons therefor by De Beurmann and Gougerot, it is evident that more precise work with a larger number of cultures than has yet been available with certain varieties is demanded, and that the subject is still in an unsatisfactory state. Recent work by Davis[5] on "Chromogenesis of Cultures of Sporotricha" necessitates serious consideration as to the value of color production in classifying this group. He has found that if the pigmented and non-pigmented spores from a pigmented culture were isolated, the pigmented spores would give rise to pigmented cultures, the non-pigmented spores to white cultures, and furthermore that these cultures would breed true, as permanently white or pigmented cultures, even after animal passage. The most liberal treatment reduces the question of different species to Sp. schenki, Sp. beurmanni, Sp. jeanselmei, and Sp. gougeroti.

In the consideration of the classification of the Sporotrichum isolated by us, only the first three need be considered. In gross cultural characteristics, and in pleomorphism, in luxuriance of growth upon simple media, our organism resembles Sp. jeanselmei most closely, though it shows a much greater tendency to aerial growth of the filaments. Microscopically, in the absence of lateral clusters of spores, it is different from all three species. In luxuriance of spore production it corresponds with Sp. beurmanni. In fermentation tests it does not agree with any of the results recorded by Blanchétière and Gougerot. The form of the organism in lesions, that of branching filaments, is wholly different from any other sporotrichum. All accounts of Sp. schenki, Sp. beurmanni, and Sp. gougeroti in tissues describe a short oval spore-like body. Davis[4] has given particular attention to the morphology of Sp. schenki and Sp. beurmanni in tissues and finds no essential differences between

them. He does not regard the tissue forms as spores. The tissue forms according to Davis, multiply by a process of budding. He has obtained a similar form of sporotrichum in cultures grown anaerobically and in tissues.

As has been stated above no assistance in classification is to be obtained from fermentation tests. The behavior of our organism in milk in liquefying gelatine and blood serum, and in the failure to produce indol and to reduce nitrates is in accord with the behavior of most sporotricha. The results obtained by others with complement fixation tests and agglutination tests have given no indication of value for the differentiation of sporotricha. De Beurmann and Gougerot[1] find that other mycoses give positive complement fixation tests with sporotrichum antigen. The agglutination test is said to be more specific than the complement fixation test, yet it is impossible by its means to exclude certain other mycoses or to differentiate between strains of sporotricha. Davis[3] obtained similar results in the attempt to differentiate strains of sporotricha by means of agglutination tests.

Conclusions. — At the present time the divisions of pathogenic sporotricha into species and varieties must be based on gross and microscopical characteristics in cultures and tissues. Fermentation and serological tests have not yet proved to be reliable.

We believe the sporotrichum isolated by us to be sufficiently different from other pathogenic sporotricha to warrant placing it as a separate species for which the name Sporotrichum councilmani is proposed.

The important distinguishing features of Sporotrichum councilmani are: (1) its pleomorphic growth, characterized by a free aerial growth of hyphæ; (2) the abundant spore formation, large size of the spores and absence of lateral spore clusters, and (3) the occurrence in lesions as septate branching filaments.

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DESCRIPTION OF PLATES XXXIV.–XXXVII.

Plate XXXIV., Figs. 1 to 7 illustrate details and variation in spore formation described by Dr. Meier in text. Drawing made from growth observed in van Tiegham cell.

Fig. 7a. — Beginning spore formation showing a sporophore which has become papillate. A later stage is shown on the opposite side at b.

Fig. 2b. — Shows elongation of the sporophore along line of axis (the beginning of a second spore) pushing aside a spore previously formed.

Fig. 6. — Shows the denticulation of the sporophore left by spores which have dropped off.

Figs. 1 and 4. — Spores formed directly by lateral budding from a vegetative hypha. Atypical.

Fig. 3. — An atypical arrangement of sporophores.

Fig. 5. — Terminal and lateral sporophores. Typical.

Plate XXXV., Fig. 8. — Spikelet form of growth, x 2, on glycerine agar plate, six days old. Compare with Fig. 11. Incubator temperature.

Figs. 9 and 10. — Moist pigmented and non-pigmented growth on potato, x 2. Six days old. Incubator temperature.

Fig. 11. — Flat form of growth with deep black pigmented center, on glycerine agar plate, x 2. Six days old. Incubator temperature.

Fig. 12. — Free growth of hyphæ almost filling test-tube. Pigmented spores at the bottom shown by black margin. Five weeks' growth at room temperature on potato. Slightly reduced.

Fig. 13. — Six days' growth on potato, incubator temperature, x 2. Shows a dry powdery surface of spores, mouse gray in color.

PLATE XXXVI., FIG. 14. — Growth at incubator temperature from tissues of rat, x 450 diameters. A chlamydospore at a; six weeks' old culture.

FIG. 15. — Terminal and lateral sporophores. Culture six days old, on glycerine agar. Incubator temperature, x 450 diameters.

FIG. 16. — Atypical spore formation, x 450 diameters. Compare with Plate XXXIV.

FIG. 17. — Same culture as Fig. 14. A chlamydospore at b. A "reserve cell?" at a.

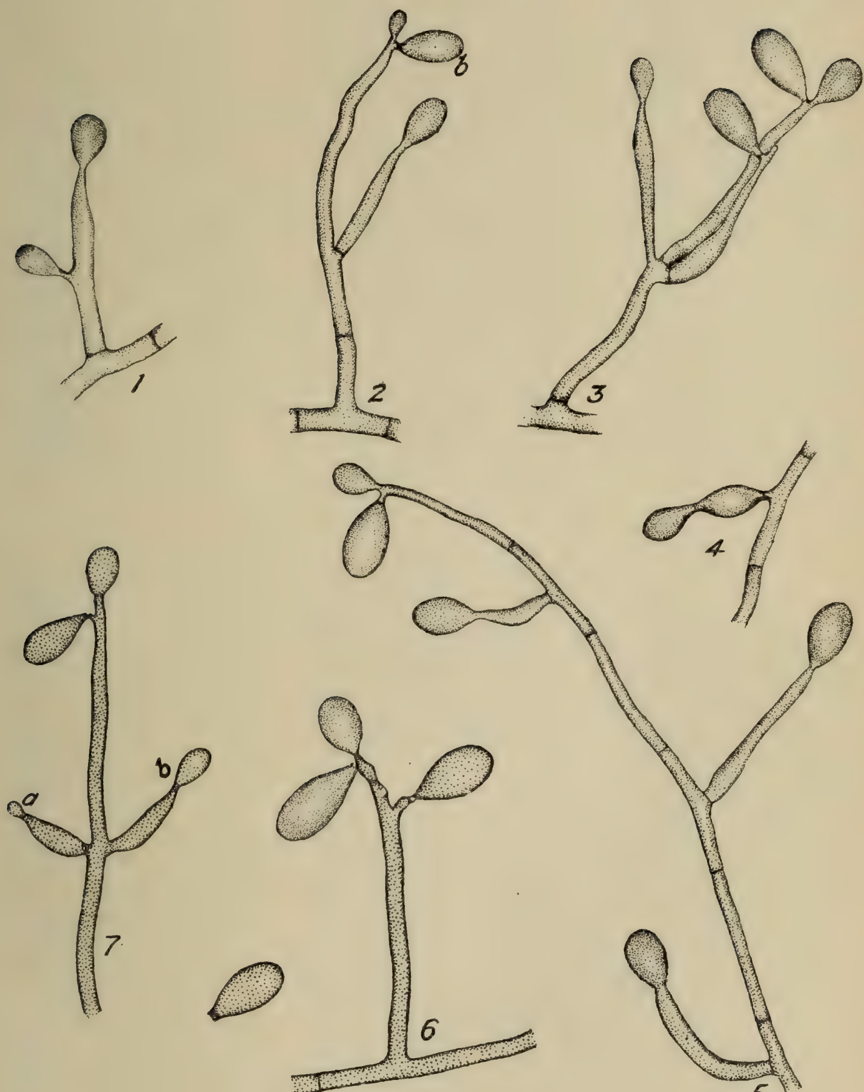
FIG. 18. — Shows spore clusters from an old agar plate culture. Unmounted preparation.

PLATE XXXVII., FIG. 19. — Higher power of Fig. 18, showing terminal spore clusters on lateral sporophores.

FIGS. 20 and 21. — Spore clusters. Six-day glycerine agar culture, x 450 diameters.

FIG. 22. — A colony of hyphæ in a miliary abscess in kidney of a white rat. Intravenous inoculation. Mallory's connective tissue stain, x 500 diameters.

FIG. 23. — Hyphæ in a brain abscess in a white rat. Intravenous inoculation. Eosin — methylene blue stain, x 500 diameters.



Wolbach, Sisson and Meier, A new Sporotrichum.

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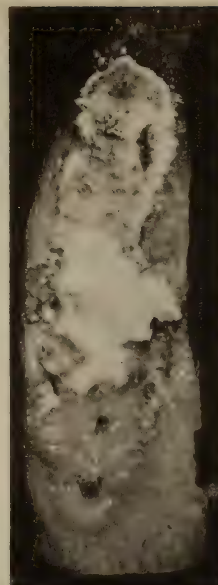

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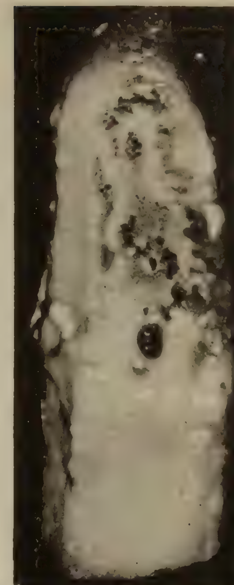

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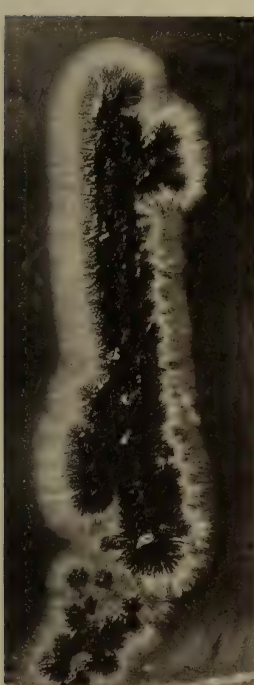

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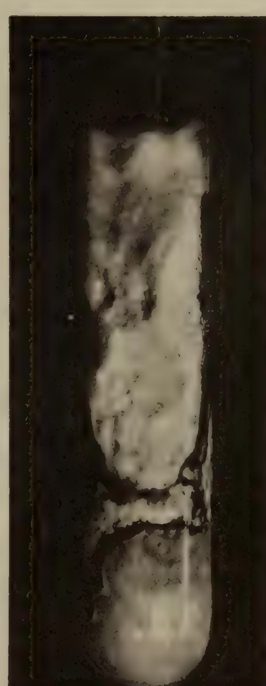

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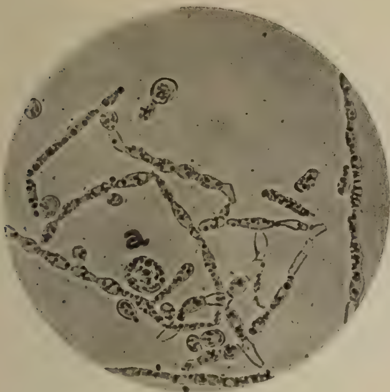

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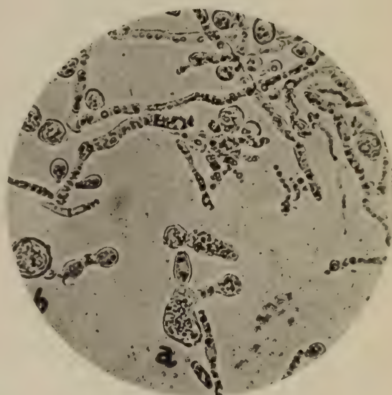

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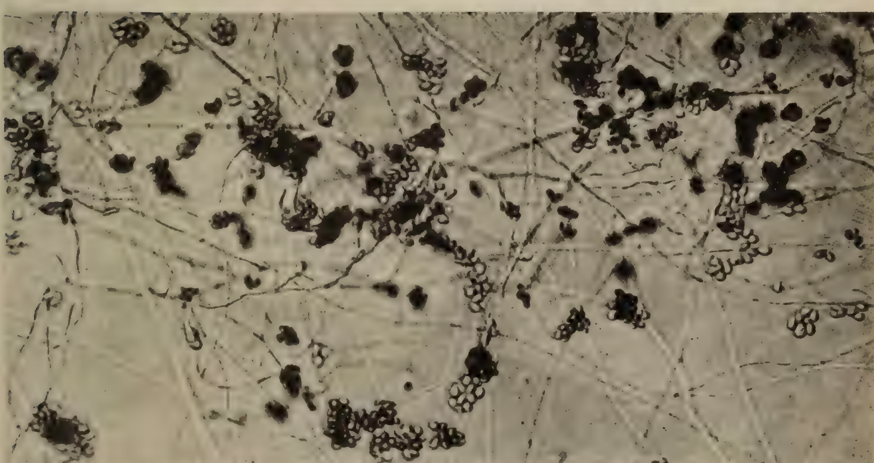

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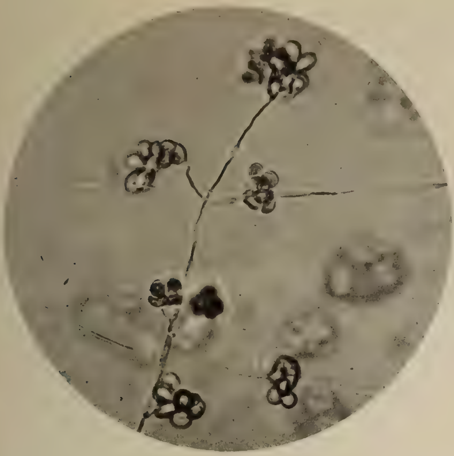

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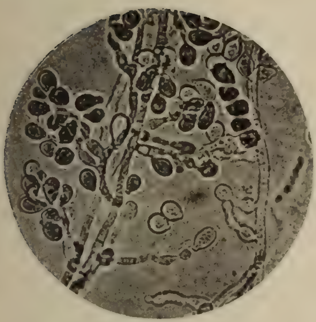

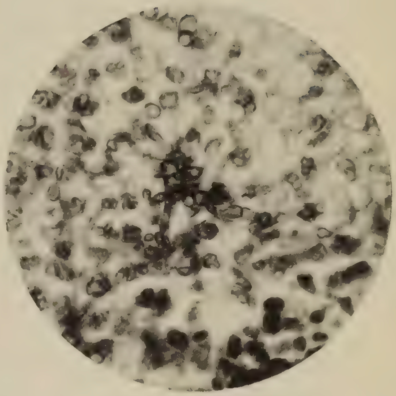

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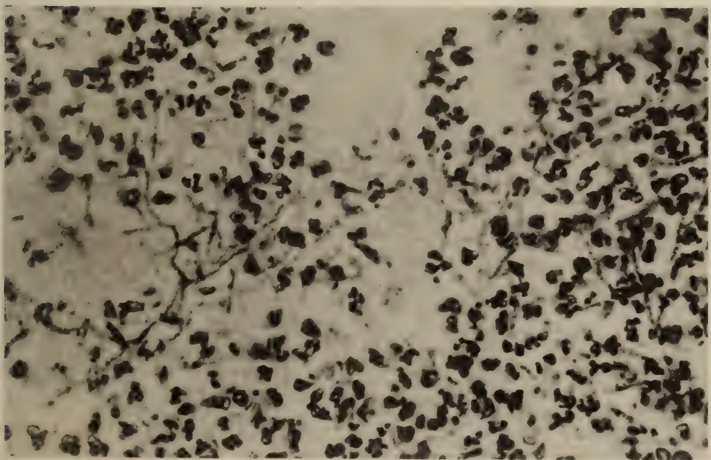

23

THE PART PLAYED BY PROTEIN-FREE DIGESTION PRODUCTS AND BY MEAT INFUSION IN DIPHTHERIA TOXIN PRODUCTION.*

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Although a considerable amount of work has been done, very little is known about some of the factors that govern the production of diphtheria toxin in culture media. This has recently been painfully realized by commercial concerns in their failure to obtain a strong toxin with peptones other than Witte's. Most of the American peptones give as good or better growth of the diphtheria bacillus than Witte's, but for some reason not yet discovered the toxin is relatively very weak.

Theobald Smith and others have worked out the influence of the reaction of the medium, of the oxygen supply, and of the presence or absence of dextrose and muscle sugar upon toxin formation; but little has been done with the nitrogenous elements of the culture medium. The various media described and advised have been purely empirical, with no exact knowledge of what nitrogenous bodies were essential or desirable for the development of a powerful toxin. Very few attempts have been made to ascertain whether these essential constituents are the amino acids, peptones, some of the various types of proteoses, or the extractive bodies.

Several workers have succeeded in obtaining very weak toxin in protein-free synthetic media, and accept this as evidence that toxin can be synthesized from very simple compounds. This conclusion is open to doubt, as in the very long periods of incubation used large numbers of the bacilli would die and undergo decomposition, with the formation of bodies from which it is possible the minute amounts of toxin found could be formed. The present work is with protein-free and protein-poor media, but is only preliminary to further researches.

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Historical review. — There has been a wide divergence of opinion as to the chemical nature of diphtheria toxin. Roux and Yersin thought it was an enzyme, Frankel and Brieger an albumin, and Wassermann and Proskauer an albumose. Dzierzgowski and Rekowski put forth the theory that the toxin was some kind of a chemical union between certain bases (as NH_3) and the albumoses. This combination they thought was analogous to that found in alkali albuminates The theory was based on the fact that no toxin was formed until the culture became alkaline by the splitting off of NH_3 and other bases from the alcohol soluble fraction. The albumose fraction in the peptone water culture remained unchanged, in so far as could be determined by the ordinary chemical tests, but they thought it was rendered toxic by a molecular rearrangement or by a loose union with certain organic bases. They isolated one organic base that was somewhat toxic.

Such a diversity of opinion would indicate that little is really known as to the chemical nature of diphtheria toxin. Conflicting results are no doubt due to the presence of impurities, for it is almost certain that the toxin has not yet been isolated in even a relatively pure state.

Guinochet and also Uschinsky grew B. diphtheriæ on a medium of protein-free urine and obtained cultures that killed guinea-pigs when injected in very large doses. Uschinsky, in addition, used a simple synthetic medium which he found was improved by the addition of urea or uric acid and cane sugar. The bacilli had the usual appearance. The cultures were incubated two to four weeks, and 8 cubic centimeters constituted the M. L D. When 1 per cent peptone was added to the medium, 1.5 cubic centimeters proved fatal to a guinea-pig. On the protein-free media his filtered cultures gave Millon's, the biuret, and the xanthoproteic tests for proteins. He thought that toxin was elaborated in the body of the bacilli and might be related to peptones or still simpler bodies.

Park and Williams produced toxin with an M. L. D. as low as .005 cubic centimeter. They found that very little toxin was formed until the culture became alkaline; so the reaction of the bouillon was a very important factor. As acid was formed from the muscle sugar of the meat infusion during growth, it was necessary to make the initial alkalinity of the culture such that the acid stage would be as brief as possible. They made the medium neutral to litmus, then added 7 cubic centimeters of N NaOH per L. Dextrose was deleterious to toxin production if enough was present to form much acid, while 2 per cent and 4 per cent peptone gave more toxin than 1 per cent. The bodies of the bacilli at no time contained toxin in appreciable amounts.

Uschinsky in a later paper did not believe that diphtheria toxin was of a protein nature. He found that it was formed in a simple synthetic

medium, and furthermore that the fraction of the filtered culture which was precipitated by $ZnCl_2$ or $ZnSO_4$, and which gave the protein test, was non-toxic, while the supernatant liquid contained the toxin. One and one-half cubic centimeters of a four to six weeks' old filtered culture killed a guinea-pig in about forty hours. Fresh strains were very difficult to cultivate on the author's medium, but he claimed that old strains grew quite well when acclimated. Since toxin was formed in his medium, it must be, he claimed, a synthetic product, and not a decomposition product of nitrogenous substances in the peptone or meat infusion commonly used.

As meat infusion had been found to be an extremely variable factor in toxin production, Spronck replaced it by a solution of brewer's yeast. He claimed that the toxin formed was stronger, and certainly more uniform. No fermentable carbohydrates were present, so the medium remained alkaline.

Martin obtained good results by the self-digestion of pigs' stomachs in 1 per cent HCl, and preparing a medium from the digestion product.

Park and Atkinson have shown that the neutralizing value of diphtheria toxin for antitoxin is independent of its toxicity for guinea-pigs.

It was shown by Theobald Smith that in broth freed from sugar by B. coli fermentation, dextrose not exceeding .2 per cent was distinctly favorable to toxin production, that the acid formed was very soon neutralized, and that the medium became alkaline. He thought the bacilli could produce and neutralize much larger quantities of acid from pure, added dextrose than would be the case with the muscle sugar naturally present in the meat. So it seemed possible that the acid products formed from the muscle sugar were different from, and not as utilizable, as those from dextrose, or else certain unknown inhibitory substances were removed during coli fermentation. B. diphtheriæ did not form acid from glycogen He thought the peptone was the true source of the toxin, and that B. coli did not produce any substance in the meat infusion that would yield toxin. Colon-fermented broth without peptone in a dose of 1 cubic centimeter contained only a trace of toxin, in spite of good growth and film formation and final alkalinity, while the same lot with the addition of 2 per cent peptone killed guinea-pigs in a dose of .01 cubic centimeter or less. No indol was formed during the colon fermentation. Other important factors in toxin production were the oxygen supply, and the manner in which stock cultures were kept.

Hitchins called attention to the effect of sterilization on the reaction of the medium. A temperature of 115° C. for one-half hour made dextrose-bouillon from .5 per cent to 1 per cent more acid, and broth without dextrose only .3 per cent. Sterilizing in the Arnold increased the acidity nearly as much. He obtained the best results by making the initial reaction +.45, and adding sterile dextrose after autoclaving, thus getting a final reaction of about +.75. In some cases the M. L. D. was as low as .002 cubic centimeter. No toxin was produced in a simple peptone —

NaCl solution. Witte's peptone was the best for toxin production. Others contained reducible substances that increased the acidity.

Hadley tried to repeat Uschinsky's work, using his medium. Out of seventy fresh strains of B. diphtheriæ only two grew and then very slightly, requiring five days of incubation. In a medium of his own invention containing asparagin, ammonium lactate, $(NH_4)_3PO_4$, glycerine, and several inorganic salts, twenty-seven strains grew, seven of them fairly well. The solid types of bacilli which are usually non-pathogenic, always grew best, and the virulent granular types least. Urea in place of asparagin proved of little value, but when glycocoll was used several of the first cultures tried grew without delay. Five cubic centimeters of a sixteen-day-old culture in this medium killed a 500-gram guinea-pig in thirty to forty-eight hours. The organism recovered at autopsy was of a changed type morphologically in each case. He added agar to several of his best media, but the growth of B. diphtheriæ was slight. By carrying through a series of tubes he got five fresh cultures to grow and produce toxin in a medium made up of bouillon 1 and protein-free medium 210 parts.

It is claimed by Hida that of the various proteoses and peptones contained in commercial "peptones," the deutero-proteose fraction is the one necessary, or at least most favorable, for the production of diphtheria toxin.

An endotoxin of B. diphtheriæ with effects different from the extracellular toxin was discovered by Cruveilhier. When injected into the brain this consistently proved fatal to guinea-pigs in less than twenty-four hours. An autopsy showed none of the typical lesions caused by diphtheria toxin, but instead an intense congestion of the kidneys. Antitoxin did not protect the animal, showing still more conclusively that this poison was something distinct from diphtheria toxin.

Rist found that washed diphtheria bacilli when injected intraperitoneally caused a gradual emaciation and paralysis of the diaphragm with dyspnea. Antitoxin did not prevent these symptoms. Death usually ensued in seven to twelve days. There were nearly always the lesions of myocarditis, but in some cases no lesions at all were noticeable — simply a wasting away. The author thought that the tardiness of the results was due to slow diffusion on the part of this endotoxin.

Using various commercial peptones and four samples obtained by the peptic digestion of fibrin, Teruuchi and Hida showed that toxin production was dependent on the degree of decomposition of the peptone. In the sample that was most favorable, 18 per cent of the total nitrogen was titrable by the formol method (that is, the nitrogen was in the monoamino acid form); 75 per cent was precipitated by tannin, and 30 per cent by 65 per cent alcohol.

According to MacConkey and others, there is a seasonal variation in the potency of diphtheria toxin, the M. L. D. being considerably higher in summer than in winter. Quite an abrupt change takes place at the end of summer and in March. This is not due entirely or even in large

part, he thinks, to variations in the resistance of the guinea-pigs, but correlates well with the number of cases of diphtheria, indicating a real seasonal variation in toxin-producing powers.

Thompson, in a study of the effects of purified proteose and peptone fractions on the coagulation time and blood pressure, found that the proteoses had the most marked effects. When injected intravenously all the constituents of Witte's peptone save the antipeptone fraction caused a marked fall in blood pressure. The greatest decrease was produced by the protoproteose, with the heteroproteose next.

Roger also showed that the toxicity of peptones was indirectly proportional to their degree of degradation. He digested two lots of rabbit muscle, one with 3 per cent and the other with 15 per cent H_2SO_4. The former solution was found to contain peptones but no proteoses, and proved fatal to rabbits when injected intravenously, The part digested with 15 per cent acid was abiuretic and, moreover, was not at all injurious, even when much larger doses were used than before.

It was found by Besredka and his associates that B. typhosus, B. diphtheriæ, and meningococcus, when grown on peptone media, adsorb something from the peptone, which when mixed with fresh guinea-pig serum gives rise to a poison. The mixture killed guinea-pigs a few minutes after injection, but they could be protected by an intravenous injection of peptone a short time before. When the organisms were grown on non-peptone media even larger doses had no effect. They called this poison "pepto-toxin."

Knaffl-Lenz noticed that the toxicity of peptones ran parallel to their tryptophane content. Gelatine and zein peptones which contain no tryptophane were non-toxic; while of several peptones prepared from vegetable proteins cucurbitin peptone, with the largest content of tryptophane, was the most toxic. Lactalbumin and Witte's peptones, which have large amounts of tryptophane, produced effects still more marked.

It is apparent from this review that but little success has been met with in the production of diphtheria toxin in protein-free media. When evidences of toxin were found by using relatively very large doses, it was only after such a long period of incubation that there is ground for believing that the disintegration products of the dead bacilli played an important part. All the protein-free media employed were synthetic, being built up of relatively simple isolated substances of known chemical structure.

Since B. diphtheriæ grew so well in opsine and in the acid hydrolysis products employed in another investigation and fully described in another paper, it was determined to study toxin formation in these media.

Experimental. — The protein-free media, opsine, and the acid-digestion products of casein, lactalbumin, and edestin, are here described briefly as follows:

Opsine is the product resulting from the combined action of pepsin, trypsin, and erepsin on a mixture of proteins. It is a trade product prepared by M. Grémy, Paris, and is claimed to possess high nutritive value as a substitute for the more complex foods in instances of impaired digestive function. It fails to give the biuret test for proteins, but is particularly rich in monamino acids, as is shown by the formol titration method. It is obtained as a solid which dissolves readily in water, imparting an amber to port-wine color to the solution.

The acid-digestion products of casein, lactalbumin, and edestin were kindly prepared for us by Prof. Lafayette B. Mendel of the Sheffield Scientific School. Definite amounts of the different proteins were boiled with ten per cent hydrochloric acid under a reflux condenser until the solutions no longer responded to the biuret test. Much of the hydrochloric acid was removed by continued evaporation on a water bath. The remaining acid was neutralized with sodium hydroxide. The solutions were all of a dark brown color, but, like the opsine, they were readily decolorized by animal charcoal.

Opsine and the mixture of casein-digestion products were the principal media of this research. For the sake of brevity the casein products will be referred to hereafter as "Casein C."

The diphtheria organism used was Park's No. 8. This was kept on blood serum in the refrigerator and transplanted every week. Subcultures were made in the same medium as that contained in the toxin flasks. All of the media were put in one-hundred-and-fifty-cubic-centimeter Erlenmeyer flasks, twenty cubic centimeters to each, and sterilized at twelve to fourteen pounds of steam pressure for fifteen minutes. At the end of the period of incubation enough pure phenol was added to each flask to make from .5 to .75 per cent strength. The flasks were shaken well and kept at

16° C. until the bacilli were all dead (usually two to three days), when injections were made subcutaneously in guinea-pigs. Ponder stains were made, and slant agars were streaked, to determine when the bacilli had all been killed off.

The animals that received a fatal dose usually died in from one to two days. Several, however, did not succumb until from four to six days. There was always the same well-known and typical appearance at autopsy. A few others gradually wasted away until about the eighth day, when they were so emaciated and weak that they were etherized.

Experiment 1. — The following media were prepared:

I. Standard diphtheria bouillon: Fresh beef infusion, made by extracting 1 pound of chopped lean beef with 1 liter of water in the cold for 24 hours, was coagulated on a boiling water bath, filtered, and 2 per cent Witte's peptone added to the filtrate. This was heated again on the water bath for about 20 minutes, the reaction being adjusted to + .6 to phenolphthalein, then filtered and put in the flasks. After sterilization enough sterile dextrose was added to each flask to make .1 per cent

II. Opsine 1 per cent: The opsine was dissolved in water over a free flame, the reaction corrected as above, boiled two or three minutes and filtered. No dextrose was added.

III. "Casein C" 5 per cent (decolorized by filtering through animal charcoal), + Liebig's meat extract .5 per cent: These were added to water and heated on a boiling water bath about 20 minutes, the reaction being adjusted to + .6 as in I, then filtered. No dextrose was added.

The cultures were incubated nine days at 37°. The dose, which was injected subcutaneously, was in each case .5 per cent of the body weight of the guinea-pig. The cultures in bouillon and in "Casein C" (five per cent) + Liebig's extract .5 per cent proved fatal in eighteen and twenty-five hours respectively. With opsine (one per cent) there was a severe local reaction and great loss of weight, but the animal did not die. The growth in this one per cent medium was rather scanty — much less than in the other two media used — so in the next experiment a two per cent solution of opsine was tried. Here the growth was good, being nearly equal to that in the bouillon.

Liebig's extract in a one per cent solution gave a very weak biuret test, so Medium III cannot be regarded as

absolutely protein-free, although very nearly so. In "Casein C" (five per cent) alone, the strain of the diphtheria bacillus employed would not grow, or only very sparsely, hence it was necessary to add the extract.

Experiment 2. — The following media were prepared in a manner similar to those in the first experiment:

I. Standard diphtheria bouillon: Reaction + 1 to phenolphthalein.

II. Opsine 1 per cent, neutral to litmus.

III. "Casein C" 5 per cent, Liebig's extract .5 per cent, neutral to litmus.

IV. Opsine 2 per cent, neutral to litmus.

V. "Casein C" 8 per cent, neutral to litmus.

VI. "Casein C" 3 per cent, edestin product 3 per cent, lactalbumin product 3 per cent, neutral to litmus.

After sterilization, enough sterile dextrose to make .1 per cent was added to one-half of the flasks of each kind of medium. In one experiment the first four media were inoculated and given an incubation period of eight days at 34° C. instead of 37° C. All showed good growth and film, except opsine one per cent in which growth was very scanty, and therefore was not injected. Unfortunately, the standard bouillon flasks were all contaminated. The dextrose made no distinctly noticeable difference in the growths.

When incubated eight days and given in doses of .2 per cent of the body weight, cultures in "Casein C" five per cent + Liebig's extract .5 per cent, and in opsine two per cent had very little or no effect, while in opsine two per cent + dextrose .1 per cent the culture was toxic enough to kill a guinea-pig in seven days. With an incubation period of seventeen days, .5 per cent of body weight of Medium III ("Casein C" five per cent + extract .5 per cent) killed in less than four days, and .3 per cent of the same medium + .1 per cent dextrose produced a loss of weight and local reaction, but was not fatal. Three-tenths per cent of Medium IV (opsine two per cent) killed in four days, while the same + .1 per cent dextrose was fatal in less than two days.

The better results with the seventeen-day period may be due to the longer time of incubation, the larger doses used

or to both. In later work, when the opsine was made faintly alkaline to litmus instead of neutral, more toxin was obtained on eight days' incubation than in this case with the seventeen days.

Flasks containing "Casein C" eight per cent were inoculated from the third transplant in the same medium twenty-four hours old, and flasks with Medium VI from the second transplant in the same forty-eight hours old. All were incubated fourteen days at 34° C. There were fair growths in all, mostly large granular clumps, but also partial films. Dextrose did not make any distinct difference in the growth. A dose of .4 per cent of body weight was used in every case.

In spite of reasonably good growth there was no evidence of toxin formation in "Casein C" eight per cent, and signs of slight traces only in a mixture of the three acid hydrolysis products. The addition of .1 per cent of dextrose had no pronounced effect. It caused a slightly greater decrease in body weight with the "Casein C," and less with the mixture. Evidently something necessary for toxin production was lacking in these media. It was thought at first that, since these hydrolysis products were very acid, enough salt might be formed on neutralization to be injurious. But by quantitative methods the NaCl formed by neutralizing with NaOH was found not to exceed one per cent, so would hardly be at all inhibitory. The fact that with .5 per cent Liebig's extract in the "Casein C" medium enough toxin was formed to kill in a dose of .5 per cent of body weight confirmed the belief that these media were not inhibitory to toxin, but that some necessary element or elements were lacking in them. The meat extract in a slight degree supplied this. Further experiments with the addition of other substances to these media are desirable.

Experiment 3. — The same media as in the last experiment, with the exception of opsine 1 per cent, were prepared, with a reaction of + .4 in the standard bouillon, and very faintly alkaline to litmus in the others. The standard bouillon and the opsine 2 per cent flasks were inoculated from a forty-eight-hour culture in the same medium in each case, and incubated eight days at 34° C.

A dose of .3 per cent of body weight was injected subcutaneously in each case, that of the standard bouillon culture killing the guinea-pig in twenty-four hours, and that of the opsine culture in about forty-eight hours. Smaller doses of both were then tried, dilutions being made in sterile salt solution, so that from one to two cubic centimeters of liquid were injected in each animal. Doses of .02 cubic centimeter and .01 cubic centimeter of the standard bouillon culture killed 350-gram guinea-pigs in twenty-four hours with the typical post-mortem appearances. No smaller amounts were tried. With the opsine two per cent an amount equivalent to .2 per cent body weight, or in this case .7 cubic centimeter, killed a 335-gram guinea-pig in two days. The post-mortem appearance was the same as before.

The injection of an amount equal to .1 per cent of the body weight of the animal, either of the culture in opsine only, or in opsine + .1 per cent dextrose did not kill the animal, although it produced a very marked falling off in body weight, and extensive congestion and necrosis at the site of inoculation in both cases. With opsine only the falling off in weight was much the greater, and the animal was so thin and weak on the sixteenth day that it was etherized.

The flasks of "Casein C" + .5 per cent Liebig's extract (III), "Casein C," eight per cent (V), and "Casein C," 2.5 per cent + edestin product 2.5 per cent + lactalbumin product 2.5 per cent (VI), were inoculated from a three-day culture in Medium III, and this from a twelve-day culture in Medium VI. They were incubated at 34° C. for thirteen days.

In Medium III and III + .1 per cent dextrose there was good growth and a nearly complete film, in V fair growth with a film of broken patches. There was a little better growth in V + .1 per cent dextrose, and in VI fair growth with thick broken patches covering about one-third of the surface.

In the case of Medium III, .3 per cent of the body weight was injected and of the other two .5 per cent. None

of the animals died, but the one injected with Medium III showed some local necrosis. The others gave no evidence of any toxin whatever being present.

Experiments showing the Influence of Meat Infusion upon Diphtheria Toxin Production in Peptone (Witte's) and Opsine.

Experiment I.

The following media were made in the same way as in the previous experiments, all faintly alkaline to litmus:

I. Witte's peptone, 2%, NaCl .5%.
II. Fresh beef infusion, NaCl .5%.
III. Fresh beef infusion, opsine 2%.

The cultures were incubated eight days at 34° C. In the first medium there was very scanty growth, with only a granular sediment and no film; in the beef infusion there was a thin but complete film, and in the last medium very abundant growth with a thick heavy film. The addition of .1 per cent dextrose did not make any appreciable difference. A dose of .5 per cent body weight of the peptone culture had no apparent effect on a 260-gram guinea-pig. A dose of .2 per cent of the beef infusion culture killed a 335-gram guinea-pig in less than forty-eight hours with the typical lesions at autopsy. A dose of .05 per cent of body weight of the same medium + .1 per cent dextrose failed to kill, and produced only slight necrosis. As to the beef infusion + opsine culture, a dose of .01 per cent body weight caused the death of the animal in less than forty-eight hours, while one-third of this amount did not prove fatal, but caused extensive necrosis and later emaciation and paralysis.

Glycerine five per cent added to opsine two per cent gave a very abundant growth and heavy film in eight days; in fact, a more luxuriant growth was obtained than in the standard bouillon control, but a dose of .2 per cent produced only very slight necrosis in a 465-gram guinea-pig. With

the hope that repeated transfers in opsine might yield better toxin, a culture from serum was carried through six subcultures in opsine two per cent, transplanting every two or three days. This had no distinct influence, however, as a dose of .05 per cent had no apparent effect on a 265-gram guinea-pig.

Very little, if any, acid could have been produced from the glycerine in opsine, as the cultures were still faintly alkaline to litmus after three, five, and eight days.

Experiment 2.

The following media were prepared, all faintly alkaline to litmus:

I.	Fresh beef infusion + Witte's peptone 2%	+	NaCl .05%.
II.	" " " (no peptone)	+	" "
III.	(No beef infusion.) Witte's peptone 2%	+	" "
IV.	Fresh beef infusion + opsine 2%	+	" "
V.	(No beef infusion) " "	+	" "
VI.	Liebig's extract 1% (no opsine)	+	" "

The flasks were inoculated from forty-eight-hour cultures in the same medium, save III and VI, which were inoculated from I, as the growth of these two media was very sparse. All were incubated eight days at 34° C., and one drop of phenol was added to each flask.

Cultural development. — I. Thick, complete film with coarse clumps in it, and heavy, coarse, granular precipitate.

II. Very thin, complete film and fairly heavy granular precipitate. Growth considerably less abundant than in I.

III. No film, liquid perfectly clear and only a scanty, rather fine granular sediment.

IV. Heavy film, nearly complete, with many large clumps in it, and a very heavy coarse granular precipitate. Growth considerably more luxuriant than in I.

V. Film like IV, but only a few large clumps of sediment.

VI. No film, liquid quite clear with a heavy viscous precipitate which on shaking gave a uniform cloud.

All of the cultures were faintly alkaline to litmus.

Toxicity tests. — One one-hundredth cubic centimeter of the culture in I (standard bouillon) killed a 555-gram guinea-pig in a little over two days: .005 cubic centimeter killed a 345-gram animal in five and one-half days.

As to the culture in II (beef infusion), .1 per cent of body weight, which was tried first, failed to kill, but caused a marked congestion and necrosis over a large area at the site of inoculation. Two-tenths per cent did not prove fatal either, but by the eighth day the animal was so emaciated and weak that it was etherized. It also had a large, thickened necrotic area at the site of inoculation which was beginning to slough off around the edge, showing ulceration.

In the hope that the culture in IV (beef infusion + opsine) would prove strongly toxic, very small doses were used, but the results were disappointing: .01 cubic centimeter had no visible effect on a 205-gram guinea-pig, while .02 cubic centimeter presented only a slight local reaction (small scab and hair falling out) in a 305-gram animal.

One-tenth of one per cent of the body weight of the culture in V (opsine) did not prove fatal, but caused a severe local reaction in a 245-gram pig. Two-tenths per cent, which was then injected, had a more marked effect, the animal being so dull and weak on the eighth day that it was etherized.

An injection of the culture in VI (Liebig's extract) equal to .5 per cent body weight produced only a *very* slight local reaction (skin scaling) in a 275-gram pig.

To test the relative toxicity of standard bouillon and opsine two per cent, five cubic centimeters of each of these sterile media were injected subcutaneously. No local lesions were produced in either case, but the pig injected with standard bouillon showed a greater loss in body weight. After three weeks it was found dead, presumably from some other cause. No autopsy was made.

Later, two other guinea-pigs were injected, one with four cubic centimeters of sterile standard broth, and the other with 4.8 cubic centimeters of sterile two per cent opsine. The animals showed no ill effects or local lesions other than temporary responses due to the injection of such a large amount of liquid, and there was a gain in weight instead of a loss.

Before proceeding to the general discussion, the results of the toxin production in the various media may be summarized in the following tables:

Table showing the M. L. D. of cultures of B. diphtheriæ in the different media used:

Medium.	M. L. D.
Diphtheria bouillon	0.005 cc.
Opsine 2%	0.700 " = 0.2% of body wt.
Fresh beef infusion	0.640 " = 0.2% " " "
" " " + opsine 2%	0.035 " = 0.01% " " "
"Casein C" 5% + Liebig's meat ext. 0.5%	1.470 " = 0.5% " " "

Table of media in which cultures were non-toxic or nearly so:

Medium.	Dose.	Effect.
Witte's peptone 2% + NaCl 0.5%.	1.30 cc. = 0.5% of body wt.	None.
Liebig's meat extract 1%.	1.40 cc. = 0.5% of body wt.	None.
"Casein C" 8%.	1.26 cc. = 0.4% of body wt.	Slight local reaction.
"Casein C" 3% + lactalbumen product 3% + edestin product 3%.	1.56 cc. = 0.4% of body wt.	Slight local reaction.

General discussion. — In opsine only, in spite of quite rapid and luxuriant growth, there was very little toxin produced by the diphtheria bacillus. This agrees with the recent experiences of many laboratories in the use of American peptones. With these peptones there was often more luxuriant growth than with Witte's, but the toxin was always very much weaker. The most probable explanation is that, in addition to the substances favoring growth, certain other bodies are necessary in a culture medium in order that toxin may be formed. The Witte's peptone contains these toxin-producing compounds in larger amounts than the other brands of peptone. In other words, although reasonably good growth is necessary for strong toxin production, there may be quite luxuriant growth, as in the case of opsine and certain brands of peptone, and still very little toxin.

If diphtheria toxin is synthetic, as Uschinsky claimed, one would expect the amount of toxin built up to vary directly with the amount of growth. More toxin would also be looked for in media of relatively simple constituents, such as opsine and the acid digestion products, than in the complex proteoses and polypeptids of Witte's peptone, since the former media would furnish a large variety of amino acids for the upbuilding of a toxin of a protein nature. Uschinsky claimed that toxin was synthesized in his very simple chemically defined medium containing only asparagin and ammonium lactate as the source of N. If some toxin was really synthesized in such a meager medium as this, there should be many times as much toxin formed in opsine, which contains so many more nitrogenous bodies of the same or similar nature, and in which the growth is so much better. But this was not the case, the toxicity of the opsine cultures being only about twice that of Uschinsky's cultures. A more probable explanation is that the minute amount of toxin found was formed in the process of rearrangement or digestion of the bacterial protein or other complex nitrogenous substances of dead bacilli. As the growth was much more rapid and luxuriant in the opsine, this process was accelerated in that medium.

In the fresh beef infusion only there was much less growth than in opsine, but the M. L. D. was found to be the same. This may be explained by the fact that meat infusion is known to contain very small amounts of proteoses and peptones (Sullivan and also Armand de Lille), from which the toxin is in all probability formed. When opsine was added, and a greatly increased growth obtained, the toxin-producing substances contained in the infusion were increased. Thus a much more potent toxin was found in the culture.

In the process of preparing Liebig's meat extract the toxin-producing substances are evidently destroyed, as a dose of 1.4 cubic centimeters, equivalent to .5 per cent of the body weight of the animal had almost no effect. It is likely that these substances are hydrolyzed to simpler bodies in the preparation of the extract. It is interesting to note that the

appearance of the growth in this medium differed markedly from that in the other media. No film or coarse flaky sediment was formed. The upper portion was clear, but on shaking, the fine viscous sediment rose in a swirl and produced a uniform cloud.

With Witte's peptone (two per cent) and NaCl (.5 per cent), the growth was extremely scanty, and no evidence of any toxin was seen. But when the meat infusion was added there was both good growth and the maximum amount of toxin. The infusion supplies the materials necessary for growth, and the peptone those for toxin formation. Both are needed for good toxin production. Either alone yields cultures of very low or no toxicity. Further experiments in combining peptone and opsine would be interesting and enlightening. According to the theory, such a medium should give cultures of very great toxicity, as the opsine is very favorable to growth, and the peptone contains the bodies from which toxin can be formed.

As stated before, the present work is largely preliminary and suggestive. It was started in the hope and belief that a potent toxin would be formed in opsine, since B. diphtheriæ grew so much better in it than in any previous protein-free medium. But the results were not as expected, and leave little hope that enough diphtheria toxin can be formed in a protein-free medium to be isolated and studied. All of the experiments present evidence to disprove Uschinsky's theory that diphtheria toxin was synthesized in a medium from relatively simple substances, such as the amino acids. They tend rather to confirm the belief of Hida and Dzierzgowski that the toxin is formed from more complex constituents of the medium, namely, the proteoses, or perhaps the polypeptids. It is a well-known fact that Witte's peptone has undergone less decomposition, and consequently contains more of the proteoses and higher polypeptids than American peptones. On this account it yields much more toxin. Small amounts of protein-like substances are found in meat infusion. In the protein-free media the proteoses and polypeptids may have been produced in the decomposition of dead bacteria.

In the acid hydrolysis products growth was slow and much less luxuriant than in the opsine. Injections into guinea-pigs of .4 per cent and .5 per cent of body weight of these cultures gave evidences of very little or no toxin. When .5 per cent of Liebig's meat extract was added, however, growth was much more rapid and luxuriant, and enough toxin was developed to kill a guinea-pig with the same dose as used before.

Some of the symptoms described by Rist as being due to endotoxins of the diphtheria bacillus were noted in several instances. These included a gradual and marked emaciation, partial paralysis, and dyspnea. Further work with filtered cultures would be necessary to determine whether endotoxins really played an important part in these cases.

The seasonal variation described by MacConkey was also observed. The work was begun in the winter (January), and as the spring advanced it was found that in general much larger doses were necessary to prove fatal, all conditions being as nearly as possible the same.

The results obtained from the subcutaneous injection in guinea-pigs of sterile opsine and bouillon confirm Dalimier's finding that opsine is non-toxic, but were not conclusive as to the bouillon. They also agree with the experiments of Roger and of Knaffl-Lenz, since opsine is an abiuretic digestion product and does not contain appreciable amounts of tryptophane.

Further work with opsine should include experiments to determine the neutralizing value of B. diphtheriæ cultures for antitoxin, since this was shown by Park and Atkinson to be independent of toxicity for guinea-pigs. Purified proteose fractions could also be added to opsine to further test the essential part they play in toxin formation.

SUMMARY.

1. Very little diphtheria toxin was formed in opsine, a protein-free enzyme digestion product, notwithstanding rapid and abundant growth of the organism. The M. L. D. was .2 per cent of the body weight.

2. In media composed of the protein-free acid hydrolysis products of casein, edestin, and lactalbumin, growth was considerably slower and less luxuriant than in opsine, and there was almost no evidence of any toxin.

3. When Liebig's meat extract was added to the casein product, the growth was considerably improved and enough toxin was formed to kill a guinea-pig in a dose of .5 per cent of the body weight.

4. When fresh beef infusion was added to opsine, the growth was even more profuse than in diphtheria bouillon, and the toxicity was increased twenty-fold over that of cultures in opsine only.

5. In fresh beef infusion only growth was moderate and the M. L. D. about the same as in opsine.

6. Liebig's extract had undergone some change in the process of manufacture, so that no toxin was formed, although there was fairly good growth.

7. With Witte's peptone only there was very scanty growth and no toxin.

8. Sterile opsine is not in any way toxic to guinea-pigs.

9. A reaction slightly alkaline to litmus was found to be the most favorable in all of the media used.

10. In the standard diphtheria bouillon the constituents utilizable and necessary for growth are very largely supplied by the meat infusion and the substances from which toxin is formed by the peptone.

11. The evidence of these experiments tends to disprove the theory that diphtheria toxin can, as Uschinsky and Hadley claimed, be directly synthesized from comparatively simple nitrogenous substances like the amino acids. It is possible, however, that in the process of preparation of the opsine and other protein-free products important simple nitrogenous substances upon which toxin production depends were destroyed. The experiments on the contrary support the view that more complex bodies, perhaps some of the proteoses, as claimed by Hida, or polypeptids, are essential to the formation of toxin.

12. In protein-free media what little toxin is found may be formed from the disintegration products of dead bacilli during the long period of incubation. Further investigation is necessary, however, to determine this point.

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THE PHYSIOLOGICAL SIGNIFICANCE OF THE ANATOMICAL CHANGES PRODUCED IN NERVE CELLS BY THE TOXIN OF B. DIPHTHERIÆ.*

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Introduction. — During the last seven years there has been a considerable amount of original investigation in this laboratory on the changes in nerve cells resulting from many forms of stimulation, both normal, and, in the sense of being unusual, abnormal. The object of this research was to ascertain the probable correlation of the anatomical changes produced in nerve cells by the toxin of diphtheria with the changes produced by other forms of stimulation. The toxin of diphtheria was selected since its action on the nerve cells has been the subject of many investigations, and its clinical manifestations on the nervous mechanism are thoroughly recognized. Since the earliest observation of post-diphtheritic paralysis by Nicholas Lepois in 1580, establishing clinically an etiological relation between post-diphtheritic paralysis and diphtheria, much work has been done to ascertain the exact part of the diphtheritic infection in post-diphtheritic paralysis and to determine the point of initial attack of the toxin. These investigations have proved very fruitful in establishing certain constant and definite changes in the various anatomical structures, particularly those of the central and peripheral nervous mechanism.

The attempt, in this paper, will be merely to point out the changes produced in certain nerve cells by the toxin of diphtheria, and to correlate these changes with those recorded by other investigators and those produced by other forms of stimulation. These observations deal exclusively with a very highly specialized type of nerve cell, the Purkinje cell, from which most of the previous work in the classification of nerve cell activity has been drawn. I will refrain from postulating

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any theory as to the sequence of phenomena or from any attempts at the clinical application of these results except in a very general and broad way.

As a basis of comparison for the proper interpretation of the changes noted in the nerve cells after subjection to the action of the toxin of B. diphtheriæ, it is first essential to show how the reaction of a toxin may be related to the function of the cell. Bailey (1906), in his Histology, states the usual conception of the essential properties of any cell, those that are characteristic of life, as: 1, To nourish itself and grow (in cell mass) — metabolism. 2, To do work — function. 3, To respond to stimulation — irritability. 4, To move — motion. 5, To produce other cells — reproduction (growth in number of cells). The adult nerve cell has lost the capacity for division — that is eliminated. Except for those intracellular protoplasmic motions evidenced by the movement of minute granules, changes in the position of the nucleus, etc., which have to do with metabolism, motion is also eliminated. Metabolism, irritability, and function, then, are thus recognized as the vital properties to be dealt with in the case of the adult nerve cell.

Function in the usual sense is merely the outward expression of the internal activities of the cell — the translation of its potential energy into external work. It is a restricted faculty in that it is displayed only through some appropriate and conjoint mechanism of specialized cells. But this specific function only occurs when there is a suitable stimulus affecting the cell. The eye sees only under the stimulus of light; the ear hears only when stimulated by sound; the muscle contracts only when a stimulus reaches it; glands secrete only when stimulated. In short, from actual knowledge, this characteristic is so universal that it may, very fairly, be conceived to be an attribute of protoplasm; it is only to the brain, about which the least is known, that spontaneous stimulation is still credited.

Irritability is defined as that property which enables a cell to respond to external stimuli. It is only through the

property of irritability, then, that specialized function following stimulation takes place at all. In short, it is through its irritability that the stimulus to function reaches the cell.

Metabolism has to do with those activities of the cell by which nutrition is maintained. Irritability and the power to function vary directly with the state of nutrition of the cell. For all conditions bringing about this metabolic change Verworn (Irritability, page 37) has used the term stimulus. His definition is "The stimulus is every alteration in the external vital conditions." To the nerve cells, therefore, every change in environment, external or internal, is a stimulus, producing the changes of function through irritability. The only reservation is that outward function, in the usual sense, only occurs if the stimulus is brought into the normal habitual relation with the specialized mechanism of that function.

The toxin of diphtheria, while not a normal stimulus in the sense of being a usual one, certainly causes definite alterations in the external vital conditions of the cell, and is, therefore, to be regarded as a stimulus. A stimulus of any sort, whether usual or unusual, acting on an irritable cell produces certain definite changes in the structure of the cell which are recognizable cytologically. The stimulus is recognizable physiologically by outward effects of function, only if it reaches the cell in the usual way through its specialized mechanism. Nerve cells are primarily of a highly specialized type, and this specialization is one of function through irritability. All changes other than those of function are thus eliminated in the case of the adult nerve cell in respect to a biological process (Dolley, 1914). Diphtheria toxin, then, acting as a stimulus to a nerve cell will produce identical changes cytologically to those produced by usual (normal) stimulation. The diphtheria toxin does not produce outward result of function, since the stimulus does not reach the cell through its specialized mechanism necessary for function. There can be, thus, a functional reaction, a reaction of irritability, without specialized function resulting, and it is with this connotation that the term function is used in this paper.

As regards the functioning nerve cell there are two diametrically opposite possibilities of change in response to stimulation: one of functional activity and one of depression of such activity. By functional activity is meant the work of the cell; by depression, in the strict sense, the blocking or inhibition of that work. These terms, while diametrically opposite as regards function of the cell, have one point in common, namely, that the end result of either process is a complete cessation of function of the cell. It is, therefore, necessary, that a more exhaustive differentiation of these two phenomena be made.

Verworn in 1896 clearly advanced the idea that there was a difference between the depression of function due to the accumulation of waste products within the cell and the depression of function due to the actual consumption of the cell substance necessary for life. In the case of the latter, every manifestation of function calls for an expenditure of vital substance. If the demand for this expenditure of protoplasmic substance is more rapid than the metabolism of this substance can be performed, the final result will be a total loss of protoplasmic substances necessary for the maintenance of life. The intermediate stages of such a process may be looked upon as fatigue, while the end result is one of exhaustion. The exhaustion obtained in this manner may, in the sense of incapacitating the cell for further work, be looked upon as a depression. This complete depression is an exhaustion due to functional activity; but, on the other hand, as in the case of the accumulation of waste products, there may be a complete stoppage of function without substantive loss. The latter case, then, is a depression of functional activity in the more usual sense.

To summarize, exhaustion resulting from functional activity may be regarded as an inability upon the part of the cell to carry on its normal function, which is due to the actual destruction of the protoplasmic material necessary for this activity. On the other hand, depression proper may be considered as a stoppage of functional activity, or in its intermediate stages a decrease of the normal intensity of the vital

phenomena, a decrease due to the blocking of the cellular processes as by the waste products of metabolism. However, both produce the same result in final effect on outward function, that is, in external work.

But, as Verworn points out in his General Physiology, different stimuli, or even different degrees of the same stimulus, may produce first a certain degree of functional activity and later produce a depression of this function. He cites morphine as an example of a stimulus which does this. Morphine given in small doses always produces first an excitation of the body, but later the excitation disappears and deep sleep follows. The effect of the narcotics can be demonstrated on the cell as well as on the entire organism. This relation, however, can only be demonstrated by certain kinds of stimuli, for there are those that induce only functional activity (*i.e.*, pure excitant stimuli) and others that induce only a depression of this function (*i.e.*, pure depressant stimuli).

The entire nature of stimuli, in so far as they affect the nerve cell, can be briefly summed up under three heads: first, those purely excitant; second, those purely depressant; and third, those which first excite and later depress.

Verworn further demonstrates that those stimuli which consist in an increase in the environmental conditions of the cell, such as increase in the surrounding temperature, produce first the change of functional activity, and after carrying the increase to a certain point superimpose the changes of depression on this functional activity. He also shows that a diminution of the environmental conditions, for example, a decrease in the surrounding temperature, appears in general to depress with increasing intensity the vital phenomena without previous excitation.

That there are definite anatomical changes produced in the nerve cell which correspond to these physiological conditions of the cell appears demonstrated by the work of Dolley and his associates. The classification of these anatomical changes, as used in the interpretation of the changes noted in this diphtheria material, has been published several times and it

will suffice at this time to merely summarize this classification and refer the reader to the earlier publications of Dolley for their more minute description and discussion. For sake of clearness, the classification of the anatomical changes of functional activity, that is, the response to excitant stimulation will be considered first.

This classification, which has been divided into thirteen stages, is based fundamentally on the shift of chromatin in the cell and the size change in the cell. Briefly stated, in terms of the chromatin distribution, the thirteen stages are as follows (Dolley, 1910):

"1. The resting cell. — It is lacking in intranuclear chromatin except within the karyosome (nucleolus) and the amount of extranuclear chromatin varies with the individual.

"2. The stage of progressive hyperchromatism, in which the initial enlargement of the whole cell reaches its maximum.

"3. The stage of maximum hyperchromatism, which is associated with the beginning of shrinkage.

"4 and 5. The stages of regressive hyperchromatism together with the maximum of shrinkage. Coincident in place but separated originally to denote difference in shape, Stage 4 being more attenuated and spindle. Both Stages 4 and 5 are to be further divided into an early, the pure Hodge type ($4'$ and $5'$), and a late division, characterized by the sharp beginning of nuclear edema ($4''$ and $5''$). (Since 4 and 5 are identical in significance, Stage $5'$ and $5''$ represent the group.)

"6. The return of the cytoplasmic chromatin in its continued reduction to the average normal level. This stage is principally distinguished morphologically by the maximum disproportion in the size of the nucleus owing to its much greater edema.

"7 and 8. The stages leading to the primary disappearance of the cytoplasmic chromatin.

"9 and 10. The stages of secondary restoration of cytoplasmic chromatin. The chromatin is first piled about the nuclear membrane and then passes out.

"11. The stage of secondary disappearance of cytoplasmic chromatin. With the complete using up of the nervous supply, the karyosome is left containing the only vestige of basic chromatin in a much more exhausted looking cell.

"12. The disintegration and passing out of the ultimate content contained within the karyosome.

"13. The exhausted cell."

The interpretation of these stages has been made in terms of Hertwig's nucleus-plasma relation theory and his theory as to the origin of the chromatin, which is closely dependent. The nucleus-plasma theory is one of constant mass relations of nucleus to cytoplasm under normal conditions. The theory of the formation of chromatin, briefly stated, as it may be applied to the nerve cell, is that the so-called Nissl substance, — that is, extranuclear chromatin, is obtained through the activity of the nucleus. First, the cytoplasm must absorb certain material from its surroundings, the blood and lymph, and prepare this material for the nucleus. After this is accomplished the material is taken into the nucleus, where it is synthesized into chromatin, or chromatin-yielding substance. This chromatin substance, or its derivatives, are then resorbed by the cytoplasm and thus stored for quick use by the cell during activity.

Based upon this idea, the whole process may be simply separated into three main divisions: First, the stage of hyperchromatism results from the stimulus to work. The hyperchromatism declines due to the process of metabolism being slower than the cell demands under continuous stimulation. The second division is the attempt of the nucleus to compensate and to supply the cytoplasm with further material. This necessitates a relinquishing of some of the reserve material of the nucleus, which while it serves to supply a temporary demand for more chromatin, eventually weakens the nucleus so that the metabolic process is still slower. After this supply of chromatin is used up, the nucleus makes its third and last attempt to furnish material by giving out the chromatin which it holds in its karyosome. This third supply of chromatin, after being used up, leaves the cell exhausted; its supply of nuclear chromatin used up, its nucleus markedly weakened and the entire cell incapacitated for further functioning.

The progressive enlargement of the cell in the stage of hypochromatism is only to be conceived as a functional hypertrophy. With an undiminished demand for chromatin during functional activity of the cell, the cell body is forced

to take in an increased amount of material from the outside, and the nucleus, in turn, is forced to take in more prochromatin from the cytoplasm. The mechanism of the resulting hypertrophy is explained by Hertwig's nucleus-plasma relation theory. This enlargement of the cell furnishes a valuable index as to the stage of activity of a cell at any time.

Briefly summarized, the factors upon which the anatomical diagnosis of conditions of functional activity are based are: first, the shift in the amount of chromatin within the cell from hyperchromatism to hypochromatism and on to complete loss of chromatin in exhaustion; second, the shifts in the nucleus-plasma relation. In the hyperchromatic stages this is essentially a series of shifts in favor of the nucleus; later, however, the final shift is in favor of the cytoplasm and remains so until final exhaustion.

The process of depression of function is a very different anatomical condition. Depression of function, as the name implies, is merely a stoppage of the physiological function of the cell at any stage of activity. In the study of the nerve cells of any animal in depression one is able not only to find depression of the resting cells, but also to find and diagnose depressed cells of the various stages of activity. For the sake of brevity the anatomical changes of depression for the stages of activity will not receive individual attention, but the more general effects upon the cells will be noted.

The fundamental difference between a cell in a certain stage of functional activity and the same cell in depression is that in the latter the nucleus is found to contain varying amounts of chromatin, corresponding to the degree of depression, while the cytoplasm is deficient in chromatin to a corresponding degree. In the resting cell, which normally shows an abundance of extranuclear chromatin, this extranuclear deficiency varies from an appreciable lessening in amount to an absolute disappearance. The same is also true for the hyperchromatic cells, which normally have an excessive amount of extranuclear chromatin. Sharply-defined granules gradually became less distinct and the acid-staining

elements of the cytoplasm take the acid stain sharply and there is little or no basic-staining substance left. In the intermediate stages, just before the final loss of basic-staining substance, the stained cytoplasm may appear murky, turbulent, or floccular, or the remaining basic-chromatin may appear as fine dust-like particles.

The same changes noted in the hyperchromatic cells are also noted in the hypochromatic cells, but owing to their normally lessened amount of chromatin, it may not be so obvious. The marked edema present in the more advanced stages of hyperchromatism also change the appearance of the cytoplasm from a deep acid-staining hyaline mass to a much lighter staining and more broken-up mass.

The changes in the nucleus deserve a brief consideration, too, since these changes are not less marked than those apparent in the cytoplasm. In the early stages of depression of function, the nucleus-plasma relation is disturbed in favor of the nucleus. With the nuclear enlargement there is associated a progressively greater deposition of chromatin within the nucleus. This increase of the intranuclear chromatin in depression is most characteristic, since for the most part the normal nucleus contains no basic chromatin except in the karyosome. The disposition of the chromatin may be in the form of well-defined granules, or it may be in a more fluid form, giving the nucleus a more homogeneous basic-staining appearance. The karyosome may be duplicated by the appearance of like bodies until in the more advanced stages many such bodies may be present. In the last stages of depression, karyorrhexis, or a dissolution of the karyosome may appear, leaving the true, acid-staining nucleolus. The appearance of the entire nucleus, at the time of complete depression of function, is one of a homogeneous, formless mass, taking predominantly the basic stain as opposed to a more granular, mesh-like, and acid-staining appearance in the cytoplasm.

The appearance of deposits of albuminous material and glycogen in the cell in profound depression, as shown by

Dolley (1913), has a marked significance, since it demonstrates the fact that the cytoplasm is incapacitated for work to such a degree that the raw material taken in is not synthesized into prochromatin, but deposited in the cytoplasm unchanged.

Briefly, then, to summarize the changes occurring in the nerve cells due to the depression of function, they are:

(a.) A lessening in the amount of chromatin in the cytoplasm and an increase in the amount of chromatin within the nucleus, which signifies a shift in the nucleus-plasma relation in favor of the nucleus. This is evidenced by an increase in the affinity of the cytoplasm for the acid stains and an increase in the nucleus for the basic stains. The Nissl bodies become broken up (chromatolysis) giving the appearance of mechanical "powdering" of this substance. The basic substance, both in the cytoplasm and in the nucleus, becomes more homogeneous; the appearance in the cytoplasm is that of a very finely granular substance, while in the nucleus it appears as a solution of the chromatin.

(b.) A deposition of unsynthesized food material in the cytoplasm. This is shown by an excessive deposit of glycogen and albuminous material.

(c.) At the maximum a breaking up of the chromatin of the nucleus, karyorrhexis and karyolysis, a fading and disappearance of the nuclear membrane and finally a destruction of the cell. At this point the cell stains homogeneously throughout and has a hyaline appearance. The process here becomes a process of degeneration.

On the basis of such distinct differences, the changes of activity and depression, the functional state of the animals was diagnosed after subjection to various doses of different preparations of diphtheria toxin. Differential counts were made, both of the normal animal and of those subjected to the toxins, and by comparison the degree of activity or depression, either singly or together, was demonstrated.

Source of material.—The rabbit was used exclusively in this series of experiments. Belgian hares and white rabbits were used both as control animals and in the experiments and since no difference could be determined either in their behavior to the diphtheria toxin or in their normal cell counts, the results obtained readily lend themselves to comparison. Only healthy adult animals were used. The age of the animals could not be established with accuracy, but an effort was made to use only the young adults, since senility is known to produce very definite and marked changes in the nerve cells (Hodge, 1894; Dolley, 1911; Kurtz, 1915). So far as could be determined they were all exposed to the same physical conditions and given the same care after inoculation. No anesthetic was used for the inoculation since (prolonged) anesthesia has been shown to produce anatomical changes in nerve cells (Butler, 1916). The animals were all killed with ether at or before the onset of paralysis.

In order to obtain the approximate normal for rabbits under the existing conditions, a series of eight undisturbed animals was first killed by ether and the cerebellum recovered and immediately placed in the fixing fluid. The tissue from these normal animals was studied and drawings of the various stages of activity were made in order to furnish a permanent guide for the differential counts. Differential counts were made from three of these normal animals for comparison with the counts made from the animal injected with diphtheria toxin (Table 1). A second group of eleven animals was submitted to the same physical conditions as regards food, temperature, and exercise as the first group. Each animal of this group was injected with a one-plus lethal dose of a standardized toxin. Using this standardized preparation of toxin, lethal doses were calculated upon the basis that weight for weight the rabbit and the guinea-pig are equally susceptible to diphtheria toxin (Goodman, 1907). The toxin was diluted up to three cubic centimeters with physiological salt solution before injection. There were slight variations in the size of the individual doses due to

the coarseness of the graduations on the measuring instruments. The brain tissue was secured from all of these animals as soon as possible after death.

The material from four of these animals was selected for differential counting, since it seemed that they would represent the widest range of nerve cell change due to the fact that the time of the onset of the paralytic symptoms varied from two to seven days. The material from these four animals was secured in each case immediately after death of the animal, excluding the possibility of post-mortem change. Complete autopsies were made in each case to establish the fact that the animals were not suffering from an infection or some concurrent disease.

The material from the remaining animals of this group was examined from time to time to confirm the findings in the tissues upon which the differential counts were made. In this way they were useful both as a check on the counts made, and also as confirmation of the results obtained.

Experiment I. — Brown rabbit was given a lethal dose of diphtheria toxin by subcutaneous injection. The animal developed paralysis on the seventh day, and was killed with ether.

Experiment II. — Brown rabbit was given a lethal dose of diphtheria toxin subcutaneously. The animal showed paralysis of the hind legs on the seventh day and died on the ninth day. The brain was secured immediately.

Experiment III. — White rabbit was given a lethal dose of diphtheria toxin into the lateral vein of the ear. Forty-eight hours after the injection the animal showed incoördination of movement in walking and refused food. Twenty-four hours later it died and the material was immediately secured.

Experiment IV. — White male rabbit was given a lethal dose of diphtheria toxin by subcutaneous injection. Showed no evidence of paralysis until the fifth day. The animal was seen four hours before its death. At that time paralysis was marked and the animal was not able to make coördinate movements. (It is very probable that the first symptoms of paralysis began eight to twelve hours before its death.) Immediately after the death of the animal the material was secured and fixed.

Technic. — Following the experimental work of Mann (1894), Flemming (1895), v. Lenhossek (1897), and the adopted custom of this laboratory, corrosive sublimate was used constantly for the fixation of brain tissue. Dolley (1911) compared the results obtained from the more common

types of fixatives and proved that the findings in the nerve cells are constant no matter what the fixative employed. Undue distortion of shape is strikingly absent after sublimate fixation. In view of the fact that so much depends upon that feature in the anatomical diagnosis of nerve cell activity, it is superior to the other fixatives commonly employed, particularly strong alcohol. The aqueous sublimate was combined with formalin according to the following formula:

Saturated corrosive sublimate	95 cc.
40% formaldehyde	5 "

After fixation the tissue was run through the graded alcohols up to eighty per cent, and then iodized. After being run through alcohol, alcohol-xylol, xylol, and xylol-paraffin solutions, the blocks were imbedded in paraffin and routine sections cut at five-micra thickness. The sections were mounted by the water method.

Following the work of the investigators above, the sections have been stained in toluidin blue. Erythrosin has been employed as a counterstain in the solution recommended by Held (*i.e.*, one gram to one hundred and fifty cubic centimeters of distilled water, to which are added two drops of strong acetic acid.)

The technic is very simple. After removing the paraffin with xylol the sections were passed through graded alcohols to water. They are then stained one or two minutes (usually one and one-half minutes is sufficient) in the erythrosin solution warmed to 40° C. After being thoroughly washed in water, they are stained in the toluidin blue solution for about seven minutes, again washed and differentiated in ninety-five per cent alcohol. For this purpose the sections are run rapidly through fifty, seventy, and eighty per cent alcohols and then passed to the ninety-five per cent in which they are allowed to remain until sufficiently differentiated. The process of differentiation is followed under the low power and stopped when the internal markings of the cell are clear. The condition of the cells in the granular layer offers a valuable index as to the exact amount of decolorization. After placing the sections in absolute alcohol for a few minutes they are cleared in xylol and mounted in Canada balsam.

This microscopic technic has been presented in some detail to emphasize the fact that the results obtained in this study have been attained through the use of the ordinary cytological methods and there has been no occasion to resort to the more unusual stains.

TABLE No. I.

Normal Rabbits.

	N-R-2.	N-R-3.	N-R-6.	Average Normal.
ACTIVITY:				
Stage 1	10	6	3	6
" 2	20	15	19	18
" 3	33	33	48	38
" 5′	87	107	71	88
" 5″	10	17	12	13
" 6	14	15	29	19
" 7	4	8	7	6
" 8	2	3	12	6
" 9	3	6	3	4
" 10	1	3	3	3
" 11	0	4	0	1
" 12	1	1	2	1
" 13	1	3	1	2
Hyperchromatic	100	62	70	77
Hypochromatic	14	20	27	20
DEPRESSION:				
Stage 1	0	0	0	0
" 2	0	0	0	0
" 3	0	0	0	0
" 5′	0	0	0	0
" 5″	0	0	0	0
" 6	0	0	1	0
" 7	0	0	0	0
" 8	0	0	0	0
" 9	0	0	0	0
" 10	0	0	0	0
" 11	0	0	0	0
" 12	0	0	0	0
Total Hyperchromatic	260	240	223	241
Total Hypochromatic	40	63	84	62
Total	300	303	307	303

TABLE NO. II.

Diphtheria Group.

	Average Normal.	Diph. Exper. 1.	Diph. Exper. 2.	Diph. Exper. 3.	Diph. Exper. 4.
ACTIVITY:					
Stage 1	6	0	0	0	0
" 2	18	0	0	0	0
" 3	38	0	0	0	0
" 5'	88	0	0	0	0
" 5''	13	0	0	0	0
" 6	19	0	0	0	0
" 7	6	0	0	0	0
" 8	6	0	0	0	0
" 9	4	0	0	0	0
" 10	3	0	0	0	0
" 11	1	0	0	0	0
" 12	1	0	0	0	0
" 13	2	4	17	7	4
Hyperchromatic	77	21	20	19	10
Hypochromatic	20	46	23	41	40
DEPRESSION:					
Stage 1	0	14	7	5	10
" 2	0	19	14	8	14
" 3	0	14	23	20	28
" 5'	0	18	25	41	43
" 5''	0	3	6	6	9
" 6	1	32	34	51	60
" 7	0	64	38	36	48
" 8	0	34	23	25	14
" 9	0	17	21	21	10
" 10	0	10	20	11	6
" 11	0	3	13	5	2
" 12	0	3	16	4	3
Total Hyperchromatic	241	89	97	106	114
Total Hypochromatic	62	213	205	194	187
Total	303	302	302	300	301

Analysis of the material. — As a basis for the analysis of the anatomical changes in the nerve cell due to the effect of the bacterial toxin used, it was felt necessary to eliminate, as far as possible, errors of observation due to an imperfect study or due to preconceived ideas as to what condition should be expected. For this reason the basis of analysis is the differential counting of the cells as given in the accompanying Tables I. and II. This method is valuable in that it affords definite and concrete figures, although these figures are in a measure approximate, partly due to technical difficulties, partly due to the difficulty of separating a continuous process into definite and fixed stages, and partly due to the variations in different areas of the material. Although the variations in different areas of the material were not great, it was an absolute rule that beginning at a definite starting point, each consecutive cell was classified either under the definite stages of activity or depression, or both, or under the more general classes of hyperchromatism or hypochromatism if not fully identified. Since the tables present a detailed comparison of the results obtained from the differential count, only the general deductions of importance will receive consideration at this point.

For the most part, only the changes of depression are to be seen in the material obtained from the experiments. While there are a certain small number of cells found in recovery, and a smaller number of frankly senile cells present, they were so conspicuously absent in the majority of cases that a close tabulation of them was not deemed necessary. In any process of activity or depression where the stimulating factor is continuous, as in this toxic stimulation, the time is not afforded the cell for recuperation which is necessary in order to produce frankly senile cells or true types of recovery cells.

It is important, both for the sake of comparison and from the physiological side, to note that only one cell was seen in all of the material studied from normal resting animals, which could be diagnosed as a cell in depression. This was a cell in the first stage of hypochromatism (Table I). By far the

larger percentage of the cells counted were found to be of the hyperchromatic type, demonstrating the fact that under normal conditions in the undisturbed animal the nerve cells are but slightly stimulated to function.

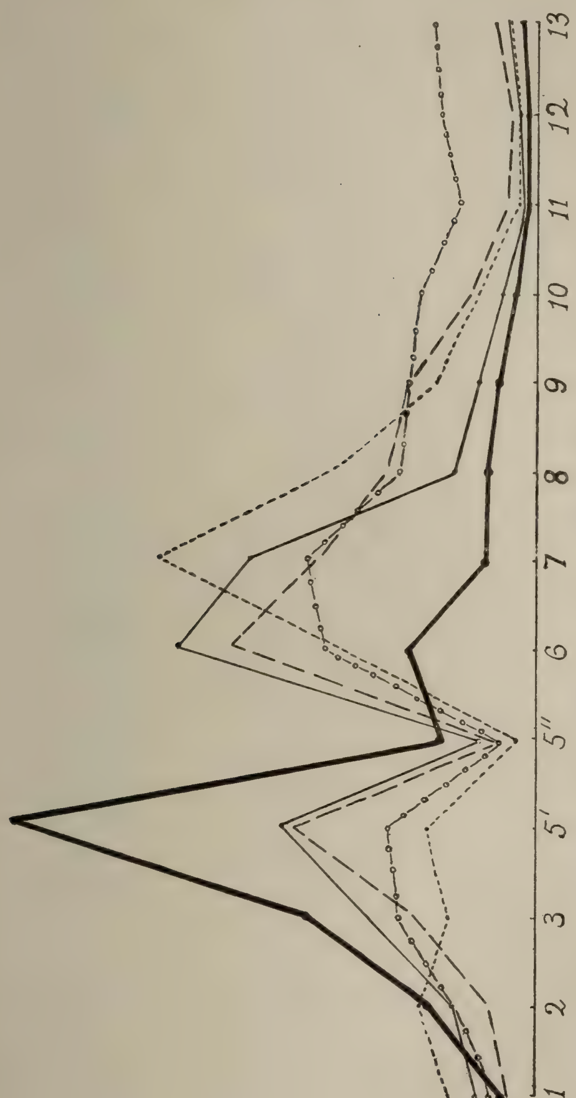

FIGURE I. — Comparison of functional activity by stages. The heavy continuous line represents the average normal activity; the more delicate lines represent the activity of the toxin-injected animals.

Text Figure I. presents a graphic representation of the average cell count from these normal animals, and compares it with the cell counts from the animals subjected to the diphtheria toxin. The average cell count from the normal animals is represented by the heavy continuous line, while the counts from the experimental animals are shown by the more delicate lines.

The graph of the average normal shows an abrupt rise from Stage 1, a maximum at Stage 5′, a rapid drop to Stage 5″, and then a more gradual decline to Stage 13, or final exhaustion.

However, counts made of the material obtained from the animals subjected to the diphtheria toxin show a more advanced activity. Plotted graphically, the curves rise from Stage 1 to Stage 5′, fall rather abruptly to Stage 5″, again rise to a maximum height between Stages 6 and 7, and finally fall gradually to Stage 13, — final exhaustion.

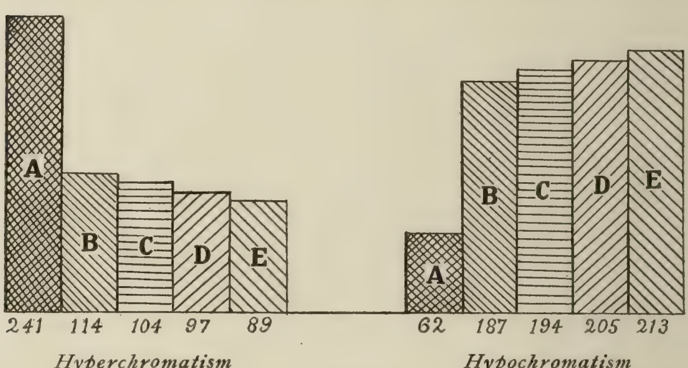

FIGURE II. — Comparison of the hyperchromatism and hypochromatism of functional activity: A, the average in normal animals; B, C, D, and E, the experimental animals.

Text Figure II. represents graphically in another way the advance in functional activity in the experimental animals due to the action of the diphtheria toxin, and compares it with that of the control animals. The blocks comprising the left-hand group represent hyperchromatism, while those

in the right-hand group represent hypochromatism. The first block in each group, lettered A, stands for the average of the counts made from the group of normal animals. The others, lettered B, C, D, and E, respectively, stand for the cell counts from the experimental animals. It will be readily apparent from the figure that in the control animals about four-fifths of the entire number of cells studied were found to belong in that general class of hyperchromatism, while the remaining one-fifth belonged in the general class of hypochromatism. This relation does not exist in the animals injected with the toxin. The counts from these animals only show approximately one-third of the cells in the hyperchromatic group, while the remaining ones show an advance in functional activity to the hypochromatic group. Reference to Text Figure I. will demonstrate this relation by stages and will also demonstrate that while activity is more advanced in the experimental group of animals, still this functional activity does not reach exhaustion. There has occurred an abnormal stimulus to work, which if continuous and uncomplicated, would result in exhaustion from functional activity, as has been observed following overwork and surgical shock (Dolley, 1909a, 1909b, 1910; Kurtz, 1915). However, the stimulus, in the case of the toxin considered, does not offer this continuous and uncomplicated excitation to functional activity. It may offer first an initial stimulus to functional activity and later introduces the second and more active feature of functional depression. The evidence from the differential counting of the cells would seem to support just such a view, namely, that there is first an initial stimulation to functional activity, but as the toxin surrounding the cells became more concentrated the stimulation becomes a depressant one. Accepting this view, the stimulus offered to the cell by the toxin of diphtheria would be considered as a mixed one, that is, first stimulating and later depressing.

However, this toxin might also be considered as a purely depressant stimulus. The more advanced activity demonstrated in the differential counts might well be the result of

the usual physiological activity of the animal. Hodge, in 1894, in his experiments proved that the nerve cell in bees at the end of the day showed a much more advanced activity than did those of the bees in the morning after a night's time had elapsed for recuperation. These results are certainly in accordance with the results to be expected. After the almost constant stimulation to physiological functional activity during the day the cell would be expected to show a certain amount of fatigue at night. Now if the cells are in a stage of early depression at the time of this activity they will not possess the normal recuperative power so that it might well be expected that during the resting period they will not recuperate to the usual extent. Then, at the beginning of the next period of functional activity, the cell will begin this activity with some fatigue already present. This, continued for several days, might well give the cytological picture found in these animals injected with diphtheria toxin. Evidence is not at hand to prove this hypothesis and so it is merely presented as a possibility at this time.

Disregarding the theoretical consideration as to the cause of the existing functional activity with the superimposed depression, the fact remains that these two factors are present and also that the depression is by far the more powerful of the effects of the toxin of diphtheria on the nerve cells.

A classification of the degrees of depression has not been found practical, but it is not difficult to distinguish between mild and profound depression. Practically all of the cells in the material from the animals injected with diphtheria toxin would be classed as in profound depression.

It is apparent from the differential counts, therefore, that there is an advance in activity and that depression is pronounced at the onset of paralysis. In the animal killed at the onset of paralysis (Experiment 1) activity had progressed so that the maximum curve height is shifted from the hyperchromatic group to the hypochromatic group and its maximum height of curve is found at Stage 6 instead of at 5′ as in the normal animals. Depression is marked in this case, but is not as profound as in those animals becoming paralyzed

and going on to death. The second animal (Experiment 2) lived nine days after inoculation and shows the most pronounced degree of functional activity, two hundred and five hypochromatic and ninety-seven hyperchromatic cells being found. Depression was of a most extreme type. In the last two animals the time elapsing before death, after the inoculation, was four and five days, respectively, and, as might be expected, there is but little difference in their total cell counts as regard hypochromatism and hyperchromatism. The time element alone does not seem sufficient to explain the more advanced activity in the one case (Experiment 3), since if this alone was the important factor a much more striking difference in the progression of hypochromatism between Experiments 3 and 2 would be expected. Too, if only the time element was considered, it would be expected that Experiment 4 would show the more advanced functional activity, since this animal lived two days longer after inoculation than did Experiment 3. A very significant feature, as shown by the cell counts, was that in the one where the paralysis lasted the longer period (Experiment 3), namely, twenty-four hours, more advanced activity was to be found, while in the animal suffering a shorter paralytic period (Experiment 4), namely, twelve hours probably at the most, the exhaustion from functional activity was less pronounced. Reference to the graphical representation of the stages of activity shows that while the total number of cells in the hypochromatic group is practically the same in both cases, Experiment 3 shows a more profound exhaustion from functional activity. That is, in the animal suffering the shorter paralysis, the curve lies constantly above that of the animal longer paralyzed until the second stage of hypochromatism is passed, when the condition is reversed and remains so until the thirteenth stage. These facts lead to the assumption that with the approximately equal doses of toxin, the degree of activity induced depends both upon the length of time elapsing between inoculation and the death of the animal and also upon the length of the paralytic stage.

These anatomical changes are significant of very important physiological changes in the cells. Certainly, cells in advanced activity, with such profound superimposed depression, are in no condition for effective function. As has been pointed out previously, if the stimulation continues long enough, cell exhaustion is the end result of functional activity and cell death of functional depression. With this in mind it becomes evident that in an intoxication from diphtheria toxin the only two physiological means of producing cellular incapacity are at work. The exhaustion resulting separately from functional activity or functional depression or a combination of these two will be evidenced externally by a lessening of the physiological function of that cell. Physiological function will be lessened and lessened as the processes advance until finally the physiological function of the cell will cease. Clinical evidence of this ever increasing incapacity is demonstrated in the retardation of muscular activity and the muscular paralysis which, in the experimental animals, always preceded toxemic death. An analysis of the fatal cases of diphtheria in human beings might lead to the assumption that this evidence is not always present. However, if those cases are excluded from analysis in which death results from some complication of the disease rather than from the toxemia itself, the evidence of the progressive lessening of physiological function will be universally found in the human cases.

A profound depression merges into degeneration, if the process continues, but so far it has been impossible to determine the actual point of transition from cytological study. Practically, this diphtheria material would be classed as very close to degeneration. The intensity of the outward effect would likely differ but slightly at the time, whether the anatomical lesion in the cell was profound depression or whether it was degeneration. In its ultimate result, if time is provided for recovery, these processes would differ widely, since the cell in depression would be capable of complete or partial recovery, while the degenerated one would obviously tend toward a permanent loss. This conception, together

with the anatomical findings in the experimentally produced diphtheritic paralysis, seems to indicate that the paralysis following diphtheria may be closely dependent upon the functional state of the cells of the central nervous system; so closely, in fact, that the paralysis may be considered as a sequel to the profound depression of the nerve cell. Where the paralysis remains permanently, it may be considered as a sequel to the end result of functional depression.

Review of literature. — In the review of the papers by other investigators on the cytological changes in the nerve cells due to the effect of bacterial toxins, only the diphtheria toxin will be considered. There will be an attempt made to correlate the findings of these investigators with those found in the present material and, so far as possible, to interpret their objective descriptions in the light of the more recent classification of activity and depression. This interpretation will be made in order to show that their findings were not at variance with those recorded in this paper.

The attempt has not been made, in collecting and reviewing the literature on this subject, to make it complete up to the present. This would be too great an undertaking since the literature on diphtheria and diphtheritic paralysis occupies no small place in medical literature. The need for a complete review of the literature on this subject was not evident since only a single phase of the subject is discussed. An attempt, however, has been made to include as many reviews of original observations on experimental or case material as possible since it is felt that they not only offer corroboration to the observations, but also add strength to the deductions.

There has been considerable investigation of the effect of the toxin of B. diphtheriæ on the nervous system, especially after the disease has reached the paralytic stage. Rainy (1900) reviewed the literature very completely up to that time, which has simplified the present task. So far as I have been able to ascertain, there has been but little original work conducted on this subject since the publication of Rainy's

work in 1900, but a few important observations will be quoted.

The first work on the neurological side of diphtheria was reported by Charcot and Vulpian (1862). They describe some changes in the fibers of the palatine nerve, but do not note the changes in the cells. Weber (1864) reported two post-diphtheritic cases in which there were no changes present in the nerve cells. The probable explanation for the non-observance of the changes in the nerve cells by these early writers is that their microscopic apparatus or technic was inadequate or their recognition of the changes imperfect.

Clos (1868) describes changes in the nerve cells in a case of diphtheria which he says are comparable to those produced by sectioning of a nerve axone. It has been proved by W. D. Davis, working in this laboratory, that the effect upon the nerve cell of sectioning the axone is one of depression primarily (Thesis, 1914). Then Clos must assuredly have seen cells in depression in his material. Oertel (1871) observed a condition which he describes as infiltration of nuclei and growth of granulation tissue in the anterior cornua of the spinal cord. In this paper he records no changes in the nerve cells themselves. Oertel (1874), writing in the "Cyclopædia of the Practice of Medicine," says that there is degeneration of the brain tissues and cells in the region of small blood clots which he observed formed in the brain. This point was not confirmed in the material examined by me, but it is very probable that if such clots are present the cells might be more affected in this area.

Vulpian (1876), in two or three cases, observed modifications in the anterior horn cells of the spinal cord, which he describes as being more globular than usual. He also notes that their content was unusually homogeneous and that it almost concealed the nucleus. These results are essentially like the ones observed in the present material. The cells in advanced activity are always swollen and edematous, while the cell showing depression often shows a diffuse distribution of chromatin especially spreading over the nucleus.

Déjerine (1878) published a paper in which he describes the nerve cells, especially those of the spinal cord, as becoming globular, as being deficient in processes, and as possessing very indistinct nuclei and nucleoli. To some extent the nerve cells are entirely deficient. Déjerine's description is very much like that made by Vulpian and the same interpretation will hold in this case. The material examined by me appeared to be deficient in the nerve cells in certain areas, but as no quantitative analysis was made this point cannot be definitely stated.

Quinquand (1880) confirms the work of Déjerine, reporting the finding of many cells affected, especially those in the lumbar region of the spinal cord.

Meyer (1881) describes some of the cells of the spinal cord as being swollen, granular, and to some extent degenerated. The swollen cells

were more than likely cells in late activity, while those showing granulation and degeneration were certainly depressed.

Kidd (1883) reported the finding of some of the cells of the spinal cord in a condition of atrophy. This finding was possibly due to the senility of the cells under observation. Dolley (1911) proves that the characteristic finding in senility is the markedly shrunken appearance of the cells.

Mendel (1885) reported the absence of cellular changes in the nerve cells of the brain and spinal cord of a child dead from diphtheritic paralysis. Kast (1886) reported an absence of cellular changes in one case studied by him. He noted atrophic change in many of the nerves. Bristowe (1888) also found no changes apparent in the nerve cells of a patient dying from diphtheritic paralysis. It must be remembered that this period represents a very early stage in the development of neurocytology. Nissl's substance had not yet been seen.

Following these two negative reports Eustace Smith (1889) and Gowers (1892) both gave credence to the finding of definite changes in the nerve cells in diphtheritic patients. The former, in his book on "Diseases of Children," notes that the nerve cells are probably affected, either primarily or later in the disease. Gowers, in the second edition of his "Diseases of the Nervous System," says in one place, "Diphtheria may cause acute changes in the nerve cells and the nerve roots," and in another place, "Diphtheritic paralysis is not wholly peripheral." Preisz (1892) recorded the finding of atrophied cells in the spinal cord, in a review of two cases of diphtheritic paralysis.

Enriquez and Hallion (1894) noted the destruction of the cells of the anterior horn of the spinal cord following diphtheritic paralysis. This entire destruction of the cells, according to our conception, is the final stage of depression.

Pernici and Scagliosi (1896) reported the results of an examination of the central nervous system of a diphtheritic patient. In the brain many of the cells were normal; others were somewhat markedly involved, and by Golgi's method were found to present varicose atrophy. The changes in the cord were indefinite, though many of the cells appeared smaller than normal and their protoplasm was granular. The nuclei are reported as having often presented a shrunken appearance, while some of the cells were in a state of extremely advanced disintegration. This description is one that applies in the main to cells in depression. The condition described as varicose atrophy refers to nodular shrinking of the processes of the cell. His observation of cells in extremely advanced disintegration is quite significant since in the material examined by me many of the cells were found to be in complete degeneration from depression and not a few had gone to disintegration.

Crocq (1896) summed up the results of his investigation in a short monograph on diphtheritic paralysis. His conclusions will be given at some length as they represent the general state of opinion in the year in

which his work was published. He says: "In the spinal cord the diphtheritic poison provokes very marked alteration in the gray matter; the cells become swollen, they stain badly, their nuclei and processes disappear; and the neuroglia and ependyma proliferate, the nerve cells atrophy or may disappear altogether and be replaced by sclerosed tissue. The white substance is rarely affected. Thus myelitis generally progresses slowly. In some cases, however, it may be rapidly evolved and may end in softening. . . . With regard to the medulla, the diphtheritic poison only produces some swelling of the cells at the lower part . . . the middle and upper parts remain almost normal. The cranial nerves are not affected by the diphtheritic poison. The poison produces in the rabbit a primary myelitis and secondary neuritis." He also says that the poison produces degeneration and irregularity of the contour of the nerves. This description applies well to the material studied, and as far as the objective description is concerned, it is very accurate. The material studied by Crocq was quite evidently in depression, but it should be interpreted as being in advanced state of activity before this depression was superimposed.

Katz (1897) examined the spinal cord of three children dead from diphtheritic paralysis, and recorded distinct changes in the motor ganglion cells and in the cells of the anterior horn. The changes were either fatty degeneration affecting the cells or complete death of the cells with all their processes, especially the axis cylinders. All ganglions cells of the cord were similarly affected, but not so markedly as the motor cells of the anterior horns. This description belongs to cells in depression since this deposition of fat in the cell is one of the differential characteristics of depression. The observation of degeneration is also a correct one, since we know that the final stage of depression is degeneration and dissolution of the cell.

Murawjeff (1897) made a series of observations on guinea-pigs which died from one to three weeks after an injection of a culture of diphtheria. He states that the first lesion to occur was a change in the nerve cell. He found certain cells in the cord in a state of chromatolysis, with nuclear changes and vacuolated. The condition he speaks of as vacuolation was the marked edema so characteristic of the advanced stages of activity while chromatolysis is always associated with deep depression. From this description one is led to believe that the cells observed by Murawjeff were in a state of profound depression following advanced activity. This is the status of the cells studied in the present material.

Babes (1898) observed chromatolysis, vacuolation, loss of nucleus and nucleolus, vascular changes, and increased numbers of round cells in the central nervous tissue which he studied. This description is one of cells in depression which is superimposed on activity. The nucleus never disappears in normal activity.

Woodhead (1898) examined two cords from experimental animals

treated with full doses of diphtheria toxin. In one case he noted chromatolysis and vacuolation of the cells as distinctly marked, while in the other he noted no deviation from the normal. The absence of changes in the second case is probably due to the fact that they were not of such a marked character and thus escaped observation.

Billington (1889) in his book, "Diphtheria — Its Nature and Treatment," reviews the observations of Déjerine (1878) and others, but does not discuss their significance. Déjerine, he says, noted that the affected nerve cells were swollen in some instances, in others shrunken, were indistinct, and had lost their processes, and were globular in shape. The number of nerve cells in certain parts of the anterior cornua of the spinal cord were greatly diminished in number. He also states that Gaucher (1881), Abercrombie (1881), in a review of seven cases of diphtheritic paralysis, and Percy Kidd (1885), in one case of his own and one case of Mott of Liverpool, each noted the same changes that had been noted by Déjerine at a much earlier time. The separate phases of this objective description have been discussed in connection with the observations of other writers and it will suffice here to say that this description is also of cells in depression. However, the description is different from the preceding ones in the fact that Billington notes the changes in the earlier stages of activity since he included in his description a note about the shrunken hyperchromatic cells (Hodge type), which is a phenomenon of early activity.

Rainy (1900) reviewed the consensus of opinion up to that time and added a considerable amount of information to this by a series of animal experiments. His work will be quoted at some length as it is of great importance and perhaps the most comprehensive up to this time. It is based for the most part on experiments on rabbits. He found that animals receiving large doses of the diphtheria toxin and living until the incidence of paralysis showed "a slight swelling of the Nissl bodies" and . . . "a fading of the chromatic substance." In another part of the paper he states that in rabbits receiving subacute maximal intoxication from the administration of the toxin and dying after the paralysis had set in, "some cells were slightly swollen, Nissl's bodies presented the appearance of somewhat advanced degeneration, the achromatic substance stained but faintly with erythrosin, the nucleus and nucleolus appeared to be normal and the nuclear membrane which, owing to the disappearance of much of the chromatic substance of the cell body, was often unusually conspicuous, was not crumpled. Some cells, on the other hand, were altered in a very peculiar manner. They were markedly shrunken. Nissl's bodies were not very much affected at first sight; but, considering the reduced volume of the cells, the amount of chromatic substance which it contained was probably diminished; the achromatic substance took on the stain with unusual intensity, whilst many of the group of cells exhibited vacuolations of the protoplasm in a most marked degree" (page 447).

This description is almost perfect for cells in depression, although Rainy does not apply this name to these changes nor attempt any functional interpretation. The only difference from the present findings is the gross vacuolations which he describes. I am inclined to think this is an artefact since a similar effect can be produced by imperfect dehydration. Certainly vacuoles involving such a large portion of the cell substance have never been observed either in activity or depression in any material so far obtained by this laboratory.

Rainy summarized his findings in a concise way and a quotation will be given:

"Diphtheritic paralysis is associated not only with changes in the peripheral nerves, but also changes in the cells of the cord itself. In my experiments such changes were invariably present after death, when paralysis was observed during life. Of the latter, the cellular changes were the most characteristic. They may, however, be associated with vascular ones. The changes are very definite and consist of chromatolysis to a moderate degree, in increased staining capacity of the achromatic substance for the acid stains, and vacuolation of the cell protoplasm" (page 450). In every instance, where his tissue varied from the normal, in his record of his experiments, he describes them as showing "chromatolysis" (pages 452–454).

A description bearing these distinctive features, as does the preceding one, certainly cannot be interpreted in any other light than that of depression. His observations make the writer very sure of his findings and also very sure of his diagnosis in the material under observation.

Rainy and Playfair (1900) report that they found vacuolation and early chromatolysis in the examination of the cells in the spinal cord of a patient dying of diphtheritic paralysis. This was reported as a corroboration of the findings made in the animal work. Rainy states that the results in this human case were very similar to those found in the rabbit experiments. This, then, must have been depression, too.

Gowers (1895) in his book, "Diseases of the Nervous System," writes: "If the elements of the spinal cord are treated with osmic acid, in the recent state, the motor cells are sometimes found in a state of intense granular and fatty degeneration." . . . "The motor nerve cells of the anterior cornua have been found altered, either swollen and unduly homogeneous or vitreus in aspect or smaller than normal and often with shrunken processes." He also quotes from Kidd (1883) as his

authority for the findings of extensive vacuolation of the nerve cells. All the points noted by Gowers have received consideration elsewhere as regards their significance in depression.

Northrup (1902), writing in "Nothnagel's Encyclopedia of Practical Medicine," says: "In the gray matter of the cord many of the cells of the anterior cornual group present two types of marked alteration. Some were slightly swollen. Nissl's bodies presented the appearance of somewhat advanced disintegration; the achromatic substance stained but faintly. The nuclear membrane, which, owing to the disappearance of much of the chromatic substance of the cell body, was often unusually conspicuous, was not crumpled. Some cells were markedly shrunken." A large part of the foregoing quotation was probably taken from the earlier work of Rainy, but at any rate it is essentially the same.

Sabolotnoff (1903) examined the spinal cords and spinal ganglia from twenty-four experimental animals. Some of these animals were injected with a bouillon culture of B. diphtheriæ while others were injected with the toxin. The earliest change which he describes is a diffuse chromatolysis which was present to a rather uniform degree in all the cells studied. Some cells presented an irregular contour, while in others the cell outline was regular. Many cells showed a very indistinct outline. Practically all of the cells showed a decreased affinity in the cytoplasm for the (basic) stain. Vacuoles, while not common, were seen in some cases, both in the cytoplasm and in the axones and dendrites. The cytoplasm sometimes had a softly granular appearance, then again it became homogeneous and looked like hyaline substance. This homogeneous appearance, as well as the other changes in the cytoplasm, was found to be present in the cytoplasmic prolongations (axone and dendrites). The nucleus stained diffusely and often had an irregular contour. The nuclear membrane was often indistinct and in many cells the nucleus had disappeared and gave the appearance of having been dissolved. The karyosome had a granular appearance, and in most of the cells took the stain. Displacement of the karyosome was not unusual. In those cells with irregularly formed nuclei the karyosome often appeared irregular in outline.

These observations are concise enough to make a diagnosis of depression seem obvious. Chromatolysis, lessening of the affinity of the cytoplasm for the basic stain, diffuseness of the staining throughout the cell, a homogeneous, indistinct nucleus, and a general softly granular, homogeneous, or hyaline-like appearance of the whole cell are the chief characteristics of depression. The presence of vacuoles in the material did not appear to be a uniform finding and they may have been artefacts due to imperfect dehydration of the

material. Certainly a description embodying, as this one does, such essential characteristics cannot be satisfactorily interpreted in any other way than that of functional depression.

Boulton (1903) contributed very accurate observations on the cellular changes in diphtheria, the material being obtained from thirteen fatal cases, eleven dying from progressive heart failure, due to the severity of the toxemia, and two of asphyxia. The two asphyxia cases were chosen for comparison since in both of these cases the toxemia was less severe than in the eleven cases of heart failure. The following description is furnished of the condition present in the large motor cells of the medulla:

"Both slight and gross changes can be seen in the same section, and, therefore, the probable course of events can be followed with tolerable accuracy. The slighter changes affect the Nissl granules of the cell, and, owing to the very large size and definite outline of the granules in the particular cells under observation, the changes are, in the majority of cases, very evident.

"The change apparently commences around the nucleus of the cell; the granules seem to break up or to be dissolved, and their places taken by a more or less finely granular débris (chromatolysis). This process spreads in every direction throughout the cell, and finally affects the dendrites, because later stages are seen in which the whole cell is finely granular, and has only a single layer of Nissl bodies arranged around the periphery. At this period the nucleus looks swollen, and is usually eccentric.

"The gross changes, which are probably the later stages of the same process, consist of a uniform swelling of the whole cell, and a further change in the position of the nucleus, which now appears at one side of the cell and frequently causes a bulging of the cell wall, as if it were about to be extruded. A swollen cell contrasts markedly with a normal one, especially if they happen to be lying side by side, as is frequently the case in my sections; instead of the normal concave, bold outlines of the cell, there is seen a large globular body with convex borders, and having the appearance of being blown out. Further changes than this I have not, as a rule, observed."

In another part of his paper he says: "All stages of degeneration can be made out from one to two perfectly normal cells to the most grossly degenerated cells, the latter greatly predominating in both number of cells and the extent of degenerative change." Again in his summary he says: "All stages of acute degenerative changes are seen in the same slide side by side."

Boulton's observations have been quoted at some length since they are exact and illuminating. He has given an accurate objective description of the progressive functional

activity with superimposed depression. While he does not use these terms, or, in fact, separate the composite picture into its component parts, still there is little or no room for doubt as to the physiological state of his material. Such objective descriptions confirm the present work and make its classification justifiable.

Laslett (1903) in a study of four cases of diphtheritic paralysis states that he found cells showing "powdery chromatolysis," and increased staining capacity in the cytoplasm for the acid stains, and some cell degeneration. He does not believe that the changes in the nerve cell are secondary to those in the fiber.

As to the priority of these changes, I take the ground that the changes are produced in the cell first, since definite changes can be determined in the cells before the onset of the paralysis, and it has not been proven that there are any changes in the fibers until after the onset of the paralysis. The priority of these changes is now being studied. The results obtained will be published at an early date. The description of the cells, as given by Laslett, can only be interpreted in the light of cells in depression, for although this description is brief, it is concise enough to assure one of its definiteness. The two features above mentioned (powdery chromatolysis and increased affinity of the cytoplasm for the acid stain) are characteristic findings in depression.

Starr (1909), in his "Organic and Functional Nervous Diseases," does not treat the subject of diphtheritic paralysis from the neurocytological side, to any great extent, but he points out that Murawjeff (1898) showed that the toxin of diphtheria produced chromatolysis and degeneration in the cell bodies of both motor and sensory neurones. This brief description can only be interpreted, in the light of the more recent classification of nerve cell reaction, as depression, and the term "degeneration" would call attention to the fact that the cells were in a state of deep depression.

Martin (1892a and b), Hochhaus (1892), Hasche (1895), Thomas (1898), Batten (1898), and Park (1901), all report negative cellular findings in the material examined by them. Batten, while not able to find any changes in the cells in the six cases studied by him, states that cellular changes have often been found. He states that it is his opinion that these changes, while occasionally present, are not constant, and that they have a very slight diagnostic value.

The non-observance of cellular changes by these writers, especially those working at an early date, is probably due to the lack of knowledge of cytology, for it is evident that any cell which possesses a nuclear mass is considered normal by some investigators.

H. F. Smyth (1914), in testing the effect of diphtheria toxin upon tissue cultures in vitro, says: "Growth of nervous tissue was arrested by decidedly smaller doses than was that of any other tissue. New cells developing from cultures with large doses of toxin were few in number and showed early advanced degeneration, heavy accumulation of fat droplets, blunted processes, and dense inactive or fragmented nuclei. . . . Tissues affected by toxin tend to recovery if not killed."

That this is a description of depression cannot be mistaken. While these observations were probably made upon cells of the nuclear or polymorphous layers and not on the more highly specialized cells, as the Purkinje, the pyramidal, or the cornual cell, still the objective description is clearly one of depression. That all cells, particularly those showing the higher degrees of specialization, can be depressed is postulated, based upon the work of Dolley and others on various types of cells. This observation is of added value in the study of functional depression since it eliminates many factors which might complicate the correct interpretation of anatomical findings — as primary axone or nerve-fiber change, or circulatory changes.

To summarize the consensus of opinion of the investigators on the effect of diphtheria toxin on nerve cells, with but few exceptions all who have studied these changes from a microscopic point of view have written descriptions that most assuredly belong to cells in a state of profound depression. Their objective descriptions, while varying in the exact wording, are in the main all alike in that they noted the characteristic changes. They observed in the cytoplasm chromatolysis, diffusion and powdering of the chromatin substance, increased affinity of the cytoplasm for the acid stain, deposition of unsynthesized food material in the cytoplasm such as fat and glycogen, and a shrinkage of the cell

body. In the nucleus the changes observed were as characteristic. They were increased amounts of chromatin in the nucleus usually of a finely granular or homogeneous nature, karyorrhexis and karyolysis where the process was passing into degeneration; finally a disappearance of the nuclear membrane and a loss of nuclear identity in the final destruction of the cell. Such descriptions emphasize the uniformity of the changes produced in the nerve cells by the action of diphtheria and confirm the interpretation of the present material.

SUMMARY AND CONCLUSIONS.

The attempt has been made in this paper to point out the anatomical changes produced in nerve cells by the action of diphtheria toxin and to interpret these changes in the light of the more recent anatomical basis of physiological cell activity and depression. This classification has been based upon the work of many investigators and is necessarily a comparison of the changes produced by this toxin with those produced by other forms of stimulation. There are two possibilities in regard to function for any cell — functional activity and functional depression. A cell when stimulated, then, must go through the changes of activity or depression or a combination of these two changes. Anatomically the changes are distinct and definite and can be recognized microscopically with accuracy. Diphtheria toxin, while not a usual stimulus to a cell, nevertheless produces changes which are identical to those produced by certain usual stimuli.

The changes produced by diphtheria toxin are essentially, if not solely, those of pure functional depression. The degree of depression produced by lethal doses of the toxin depends upon the time afforded for the action of the toxin after inoculation. Temporary cessation of function may result from profound depression, or if the process goes on to necrosis the damage is permanent. Clinical evidence is not lacking to prove that in some cases the damage done is irreparable and the paralysis permanent, while in other cases it

is temporary and the cells ultimately return to normal or nearly normal function.

The present observations while made upon the rabbit are not essentially different from those made by other investigators upon human material. They also conform to the general observations of others upon experimental animals. The interpretation of the significance of these changes, however, has never been made before in the light of functional activity and functional depression, and it is for this interpretation that originality is claimed.

The conclusions to be drawn from these observations are:

1. Diphtheria toxin when introduced into the body acts as a cell stimulus causing a depression of functional activity.

2. These changes are definite and are the same qualitatively as those produced by other forms of depressant stimuli.

3. The degree of functional activity which is associated with the depression of the diphtheria toxin is probably not due to the action of the toxin directly.

4. With fatal doses of toxin the degree of depression varies directly with the length of time that the toxin is allowed to act. This same ratio holds true for the advance in functional activity.

5. The profound depression noted is sufficient to account for the accompanying paralysis.

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THE SPLEEN AS A BACTERIAL FILTER.*

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Among the more evident functions of the spleen is the removal by it of foreign particles carried in the blood stream. The part which it plays together with the liver and the lungs in rapidly freeing the blood stream of microörganisms in cases of experimentally produced bacteriemia has been shown by several investigators. The reasons which have been assigned for this action have in part been based upon the unique arrangement of the vascular system in the spleen, and in part upon the activity of phagocytic cells which are so numerous in this organ. Thus Kyes[1] ascribes the reason of the selective collection of bacteria in the spleen to some extent to the mechanical filtering action of the modified vascular wall of vessels in this organ, stating that "A mechanical filtration of the organisms by the walls of certain of the blood vessels was found to be an important factor in the rapid accumulation of the pneumococci within this organ." Experiments in regard to the simple filtration of fluid through the spleen were carried out by Salaghi.[2] He compared the permeability of the spleen of an ox with that of the kidney of the same animal, perfusing these with defibrinated blood, both diluted and undiluted, and with physiological salt solution. According to him the spleen showed decidedly less resistance than the kidney to physiological salt solution and to the dilute blood. On the other hand, the permeability of the spleen decreased much more rapidly than that of the kidney as the concentration of the blood was increased. He reached the conclusion that under the conditions of the experiments the spleen mechanically behaves itself as a filter to the blood as thus modified for artificial circulation.

In a study[3] recently reported from this laboratory upon the fate of S. pyogenes aureus after its introduction into the

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blood stream of dogs the localization of the bacteria in the spleen and liver was shown, and especially in the former. In the spleen most of the bacteria had been ingested by leucocytes and splenic cells, but they were also found in the pulp apparently lying between the cells without having been engulfed by these — a picture which suggests the filtration ot bacteria by the pulp. This microscopic finding induced us to attempt to determine the mechanism of bacterial accumulation in this organ. The following experiments have accordingly been carried out upon spleens removed from dogs in the endeavor to obtain more definite information regarding this. The animals were killed by intracardiac injection of strychnin, and soon after the death of the animal the spleen was removed. The organ was placed in a dish containing physiological salt solution which was kept at about 38° C. It was then irrigated through the splenic artery with sterile physiological saline solution at body temperature until the fluid which came from the splenic vein was nearly colorless. The spleen thus deprived of almost its entire content of blood was perfused with sterile Locke's solution or bouillon to which a certain amount of twenty-four-hour bouillon culture of S. pyogenes aureus had been added. The bacterial fluid coming out of the splenic vein was collected after different intervals of time and plate cultures made from it. The pressure of the fluid which was perfused was kept nearly constant by having the reservoir containing the fluid placed thirty-four to thirty-six inches above the spleen. The whole amount of the fluid passed through each spleen varied from 400 cubic centimeters to 1,000 cubic centimeters, and the perfusion was repeated one or more times with the same fluid. Two obstacles were encountered during the experiment; one was clotting of blood within some of the small vessels, which in some cases retarded the circulation of the liquid through the organ, and the other was the escaping of some of the fluid through minute vessels at the hilum. This latter reduced the original quantity of the fluid decidedly at times. The fluid to be examined quantitatively for bacteria was diluted in some cases 100 times and in others 10,000 times with sterile saline

solution. From one cubic centimeter of this diluted fluid a plate culture was made, and the colonies were counted after forty-eight hours of incubation. At the same time control cultures were made from a portion of the original bacterial suspension which without having been perfused through the spleen had been kept during the experiment at approximately the same temperature as that of the perfused fluid. The saline solution which had been used for irrigation was also examined, and it was found that the germicidal power of this was practically negligible for S. pyogenes aureus. All vessels, tubes, etc., which came in contact with the perfusing fluid had previously been sterilized.

Experiment I. — The spleen was removed and perfused with 1,000 cubic centimeters of Locke's solution to which 5 cubic centimeters of bouillon culture had been added.

The original fluid showed:

At the beginning of the experiment	680,000 bacteria per cc.;
after 6 hours	600,000 " " " .

The perfused fluid showed:

After 10 minutes	(after 1 passage)	300,000 bacteria per cc.;
" $1\frac{1}{2}$ hours	(" 4 passages)	170,000 " " " ;
" 3 "	(" 6 ")	52,500 " " " ;
" 6 "	(" 9 ")	205,000 " " " .

Microscopic examination of the tissue showed numerous microörganisms in the splenic pulp. Many of them had been ingested by polymorphonuclear leucocytes; the number of cocci taken up by them was often considerable, at times twenty or more by a single cell. Many bacteria were also found in the lumina of small arteries. Few splenic cells had ingested any cocci.

Experiment II. — The spleen was removed and perfused with 1,000 cubic centimeters of Locke's solution to which 5 cubic centimeters of bouillon culture had been added.

The original fluid showed:

At the beginning of the experiment	1,220,000 bacteria per cc.;
after 5 hours	890,000 " " " .

The perfused fluid showed:

After 10 minutes	(after 1 passage)	1,090,000 bacteria per cc.;
" $1\frac{3}{4}$ hours	(" 2 passages)	650,000 " " " ;
" 3 "	(" 3 ")	660,000 " " " ;
" 5 "	(" 6 ")	1 680,000 " " " .

Histologically fewer bacteria than in the preceding experiment were found in the tissue. Most of them were found in small arteries; others were scattered in the pulp, and some of these had been ingested by polymorphonuclear leucocytes.

EXPERIMENT III. — The spleen was removed and perfused with 500 cubic centimeters of sterile bouillon to which .1 cubic centimeter of bouillon culture had been added.

The original fluid showed:

At the beginning of the experiment	46,400 bacteria per cc.;
after 1½ hours	46,000 " " " ;
" 2½ "	57,000 " " " ;
" 3½ "	73,100 " " " ;
" 4¼ "	82,500 " " " .

The perfused fluid showed:

After 20 minutes (after 1 passage)	25,900 bacteria per cc.;
" 1½ hours (" 2 passages)	43,600 " " " ;
" 2½ " (" 3 ")	33,400 " " " ;
" 3½ " (" 4 ")	30,800 " " " ;
" 4¼ " (" 5 ")	31,900 " " " .

Microscopic examination revealed the presence of only a scant number of bacteria in the splenic pulp, evidently due to the relatively small number of bacteria in the original fluid.

From these experiments it is evident that the bacteria contained in the original fluid showed a more or less marked decrease in number after perfusion through the spleen tissue. In Experiment I. the number of colonies after one passage of the fluid through the spleen was less than half of the original number, and after three hours' perfusion it had become about one-sixth. In Experiment II. the bacteria contained in the fluid also showed a distinct decrease, though not so pronounced as in the preceding experiment. Here the number of colonies was reduced after two passages almost to one-half of the original. Experiment III. also showed a moderate diminution of the bacteria in the fluid after four and five passages. On the other hand, both Experiments I. and II. showed a great increase of bacteria after six and five hours, respectively. This probably is explained by a loss of vitality of the tissue cells after a certain lapse of time, and by the growth of the bacteria which

would occur at body temperature unless prevented in some way. Also it is probable that bacteria which were detained by the living endothelial cells of the blood spaces during the earlier part of the experiment were later dislodged by the perfusing fluid as the vitality of the tissue decreased.

The experiments described above were made with spleens removed and tested shortly after the death of the animals. The vitality of the cells, during the first part of the experiments at least, is shown by the phagocytosis which had occurred. It has been shown by experiments made in this laboratory, but not yet published, that in the spleen which was carefully perfused with Locke's solution at body temperature an active phagocytosis by polymorphonuclear leucocytes as well as by splenic cells took place even after three hours. In order to exclude partially or completely the action of these living cells, we further experimented on spleens, the vitality of which had been more or less impaired or completely destroyed.

Experiment IV. — The spleen was removed from a dog thirty minutes after the death of the animal. After washing out the blood contained in the organ bacterial fluid was perfused (400 cubic centimeters of sterile bouillon mixed with 2 cubic centimeters of bouillon culture).

The original fluid showed:

At the beginning of the experiment	1,630,000 bacteria per cc.;
after 2½ hours	3,190,000 " " " ;
" 5 "	5,720,000 " " " .

The perfused fluid showed:

After 30 minutes (after 1 passage)	930,000 bacteria per cc..
" 2½ hours (" 1 ")	2,110,000 " " " ;
" 4 " (" 2 passages)	3,910,000 " " " ;
" 5 " (" 2 ")	4,740,000 " " " .

In the microscopic examination some cocci were found in the small arteries, and a very few in the pulp. Polymorphonuclear leucocytes or splenic cells which contained bacteria were not found anywhere.

Experiment V. — The spleen was removed from a dog and the blood contained in it was washed out with sterile saline solution. The organ was then placed in ice-cold sterile physiological saline solution for four days. At the end of this time it was perfused with 500 cubic centimeters

of sterile bouillon to which 2.5 cubic centimeters of bouillon culture had been added. The experiment was made at room temperature.

The original fluid showed:

At the beginning of the experiment	1,130,000 bacteria per cc.;
after 1 hour	1,100,000 " " ";
" 2 hours	1,010,000 " " ".

The perfused fluid showed:

After 10 minutes (after 1 passage)	1,110,000 bacteria per cc.;
" 1 hour (" 1 ")	1,070,000 " " ";
" 2 hours (" 2 passages)	940,000 " " ";
" $2\frac{1}{4}$ " (" 3 ")	1,220,000 " " ".

Microscopic examination showed only a few cocci in the splenic pulp.

The results of these two experiments were decidedly different from those of the first three. Namely, in Experiment IV., in which the removal of the spleen had been somewhat delayed, the reduction of the number of bacteria was not so pronounced as in the preceding cases. In Experiment V., in which a spleen was used four days after its removal, there was hardly any decrease in the number of bacteria to be found.

In the following experiment we attempted to find the result of filtration through the spleen of bacteria which had previously been sensitized with fresh dog serum. The results were in agreement with those of Experiments I. to III.

Experiment VI.—The spleen was removed from a dog and after washing out the blood perfused with 400 cubic centimeters of sterile bouillon in which 2 cubic centimeters of bouillon culture had been mixed. The bacteria had previously been sensitized with fresh dog serum for one hour in the incubator (2 cubic centimeters of bouillon culture in 6 cubic centimeters of fresh serum).

The original fluid showed:

At the beginning of the experiment	1,140,000 bacteria per cc.;
after 1 hour	770,000 " " ";
" 2 hours	790,000 " " ";
" 3 "	600,000 " " ";
" 4 "	800,000 " " ".

The perfused fluid showed:

After 10 minutes (after 1 passage)	560,000 bacteria per cc.;
" 1 hour (" 1 ")	100,000 " " ";
" 2 hours (" 2 passages)	370,000 " " ";
" 3 " (" 3 ")	650,000 " " ";
" 4 " (" 4 ")	580,000 " " ".

In the microscopic examination many bacteria were found in the tissue, and an active phagocytosis had taken place by the polymorphonuclear leucocytes. Splenic cells which contained bacteria were hardly met with at all.

In the following experiments the kidney of the dog was tested to determine its bacteria-filtering power in exactly the same way as the spleen was tested in the preceding experiments:

Experiment VII. — The kidney was removed from a dog and after washing out the blood perfused with 200 cubic centimeters of bouillon to which 1 cubic centimeter of bouillon culture had been added.

The original fluid showed:

At the beginning of the experiment	970,000 bacteria per cc.;
after 3 hours	2,570,000 " " ".

The perfused fluid showed:

After 30 minutes (after 1 passage)	370,000 bacteria per cc.;
" 2 hours (" 1 ")	220,000 " " ";
" 3 " (" 1 ")	460,000 " " ";
" 5 " (" 2 passages)	900,000 " " ".

In the microscopic examination many bacteria were found in the capillary vessels of the medulla as well as in those of the cortical portion. The most of the bacteria were found in the glomeruli, and often were present in large numbers in the afferent vessels.

Experiment VIII. — The kidney was removed from a dog and after washing out the blood perfused with 400 cubic centimeters of bouillon to which 2 cubic centimeters of bouillon culture had been added.

The original fluid showed:

At the beginning of the experiment	1,030,000 bacteria per cc.;
after 1 hour	1,040,000 " " ";
" 2 hours	1,110,000 " " ".

The perfused fluid showed:

After 30 minutes (after 1 passage)	510,000 bacteria per cc.;
" 1 hour (" 2 passages)	330,000 " " ";
" 1½ hours (" 3 ")	230,000 " " ";
" 2 " (" 4 ")	120,000 " " ".

A great many bacteria were found in the glomeruli. Frequently polymorphonuclear leucocytes were met with, which had engulfed several cocci.

EXPERIMENT IX. — A kidney, which after removal from a dog was freed of the blood contained in it and kept in ice-cold physiological saline solution for four days, was used for this experiment. The organ was perfused with 250 cubic centimeters of bouillon which contains 1.25 cubic centimeters of bouillon culture. The experiment was made at room temperature.

The original fluid showed:

At the beginning of the experiment	610,000	bacteria per cc.;
after 30 minutes	1,120,000	" " " ;
" 1½ hours	1,160,000	" " " ;
" 2½ "	1,590,000	" " " ;
" 3½ "	1,780,000	" " " ;
" 4½ "	1,550,000	" " " .

The perfused fluid showed:

After 30 minutes (after 1 passage)	10,000	bacteria per cc.;
" 1½ hours (" 1 ")	20,000	" " " ;
" 2½ " (" 1 ")	120,000	" " " ;
" 3½ " (" 2 passages)	30,000	" " " ;
" 4½ " (" 2 ")	0	" " " .

In the microscopic examination numerous bacteria were found in the glomeruli and in capillary vessels.

The results of the last three experiments showed a more pronounced diminution of bacteria in the fluid after its perfusion through the kidney than was found in the case of the spleen. It is also striking that the kidney tested four days after its removal reduced the number of bacteria equally as well as did the fresh kidney. From these results we can infer that the kidney is able to detain bacteria from the circulating fluid more readily than the spleen in so far as simple mechanical filtration is concerned. This finding seems to be rather surprising, inasmuch as the spleen readily stores up bacteria from the blood stream, while the kidney as shown by our previous results takes up scarcely any of them from the blood of the living animal. To explain this apparent contradiction we must conclude that the differences in the terminal blood vessels of the two organs give the advantage as a mere filter

to the kidney, but that during life the greater attraction of the cells in the spleen for bacteria (by chemiotaxis or otherwise) enables it to detain them in much greater numbers. Also that there is no doubt that the spleen tissue loses a great part of its vital activity very soon after the death of the animal or its removal from the living animal, though we endeavored in these experiments to keep the organ alive by perfusing Locke's solution or bouillon at blood temperature, through its parenchyma.

In spite of the greater filtering action of the kidney as compared with the spleen, this practically does not appear to play any important part in the living organism, unless coarse particles of clumped bacteria, which may give rise to emboli in this organ, are introduced into the circulating blood either from without or from a focus elsewhere in the body. On the other hand, it is well known that the spleen tissue in its fully active state readily stores up the bacteria from the circulating blood. In our experiments, however, the spleens were used the vitality of which was more or less interfered with, and therefore the filtering activity was correspondingly unfavorable. Finally the spleen kept in the ice-box four days showed practically no activity in this respect. These different degrees of filtering action thus apparently vary according to the vitality of the organ.

Finally, we must assume that the peculiar anatomical arrangement of the blood vessels in the splenic pulp also undoubtedly favors the detention of bacteria. This is not due to the mechanical filtration of these, but to retarded circulation in the complicated blood spaces, which facilitates direct and prolonged contact of bacteria with the phagocytic cells of the pulp.

CONCLUSIONS.

1. The accumulation of bacteria in the spleen, such as occurs in experimental bacteriemia is principally dependent upon the vital activity of the cells, and the mechanical filtration of bacteria by the spleen is not an important factor in their detention.

2. Considered from a mechanical standpoint alone the kidney may be regarded as a much more effective filter than the spleen for the elimination of bacteria contained in the perfusing fluid (Locke's solution or bouillon).

[In closing, I wish to acknowledge my indebtedness to Professor C. J. Bartlett, of the Pathological Department, School of Medicine, Yale University, for his kind and constant advice in the above work.]

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STUDY XIV.

The treatment of patients with bronchial asthma with subcutaneous injections of the proteins to which they are sensitive.*

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This paper is based on the study and treatment of forty patients with bronchial asthma all of whom were sensitive to some type of protein derived from bacteria, food, or animal hair. Patients who were sensitive to only the pollens and those who have been under observation for a short time only are not included in this paper. Those patients who failed to give a positive cutaneous reaction with any protein which was tried will be the subject of a succeeding paper.

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* This is the fourteenth of a series of papers on the study of bronchial asthma made possible through a gift by Mr. Charles F. Choate, Jr., of Boston, to the Peter Bent Brigham Hospital. The papers previously published are: Study I., Studies with Staphylococcus pyogenes aureus, albus, and citreus, and with Micrococcus tetragenus and catarrhalis; Study II., Studies with a diphtheroid organism isolated from the sputum of patients with bronchial asthma; Study III., Studies on the sensitization of patients with bronchial asthma to bacterial proteins as demonstrated by the skin test and the methods employed in the preparation of these proteins; Study IV., Studies on the sensitization of patients with bronchial asthma to the different proteins found in the hair of the cat and dog and in the dandruff of the horse and to the sera of these animals; Study V., Studies on the sensitization of patients with bronchial asthma to the different proteins in wheat and to the whole proteins of wheat, corn, rice, barley, rye, and oat. All in the Jour. Med. Research, 1917, Vol. XXXV., No. 3, 373–402 and 487–513.

Study VI., Immunochemistry of the proteins of cat hair; Study VII., Immunochemistry of the proteins of horse dandruff; Study VIII., Immunochemistry of the proteins of dog hair. All in the Jour. Immunology, 1917, II., No. 3, 227–246.

Study IX., The wheat proteins, Am. Jour. Bot., 1917, IV., July.

Study X., Studies on the sensitization of patients with bronchial asthma to the proteins in animal, fruit, and vegetable foods; Study XI., Studies on the sensitization of patients with bronchial asthma to the various pollens; Study XII., Complement fixation and precipitin reactions with the serum of bronchial asthmatics who are sensitive to the proteins of wheat, horse dandruff, cat hair, and bacteria, using these proteins as antigens and the cutaneous reaction as an index to sensitization; Study XIII., The relationship between the cutaneous reaction, serum agglutination tests, and the bacteriological examination of the sputum and of the nasal secretions in determining the part Staphylococcus pyogenes aureus and albus may play in the cause of bronchial asthma. All in the Jour. Med. Research, 1917, XXXVI., No. 2. Received for publication April 9, 1917.

For convenience the forty cases which are presented in this paper are divided on the basis of positive cutaneous reactions into six groups, namely, those who are sensitive to the proteins found in (1) horse dandruff, (2) cat hair, (3) wheat, (4) miscellaneous sources, (5) S. pyogenes aureus, and (6) S. pyogenes albus. This grouping does not mean that each patient was sensitive only to the proteins derived from that particular source, since many patients showed multiple sensitization, neither does it mean that each patient was treated only with those particular proteins because many patients were treated with proteins derived from several groups. The grouping does mean, however, that the patients gave the strongest cutaneous reactions with the proteins derived from that particular source and consequently treatment was given with those proteins until there was a reason for a change to something else.

In each patient a diagnosis of bronchial asthma is understood and in those patients which were studied in the medical wards the diagnosis is stated. Family histories are mentioned only where they have some bearing on bronchial asthma. Physical examinations, which were negative, are omitted and only the positive findings which might be of interest are mentioned. However, thorough physical examinations were made in each case. The history of each patient is presented in detail in order to bring out the seemingly little things which really have much to do with the condition and symptoms of the patient. For instance many patients with bronchial asthma are aware of the fact that exposure to cold air, changeable weather, wind and dust aggravate their symptoms and cause attacks of asthma. Many of these patients have indigestion, dyspnea on exertion, colds and bronchitis and attacks of asthma are associated with these conditions. Nervousness and irritability are common symptoms in these patients.

The treatment of each case is given in considerable detail in order to emphasize the necessity of careful dosage, the harm which may result from too large a dose of the proteins and the specificity of each type of protein and bacteria in

the treatment of bronchial asthma. In each case a short summary follows the treatment.

The cutaneous reaction has been the chief guide to treatment and in each case only the proteins which gave positive reactions are mentioned. It might be well to state that each patient is tested routinely with a large series of proteins, but it seems unnecessary to mention those which failed to give a positive test and therefore had no bearing on the case. In Studies III., IV., V., and X., the method of doing the cutaneous reaction and a description of a positive reaction for each type of protein have been given. Therefore there is nothing subjective or imaginative about what is called a positive reaction; it is an objective, measurable thing. A typical gradation in the size of a positive reaction or urticarial wheal which results when different dilutions of a protein are tested both before and after treatment with that protein is as follows:

Case 10.—G. V. Y., tested and treated with cat hair alkali metaprotein:

Before Treatment.		After 4 Months of Treatment.	
1-100	= 2.5 x 2 cm. wheal (6+)		
1-1,000	= 1.5 x 1 " " (4+)	1-1,000	= .75 x .75 cm. wheal (2+)
1-10,000	= 1.0 x .75 " " (3+)	1-10,000	= .5 x .5 " " (1+)
1-100,000	= .75 x .75 " " (2+)	1-100,000	= O
1-1,000,000	= .5 x .5 " " (1+)		
1-10,000,000	= O		

In the above table, the first column of figures shows the dilutions of the protein; after the equality sign the size of the urticarial wheal is given in centimeters and the figures in parenthesis represent the relative size of the wheal in pluses. Thus there is a definite relationship between the dilution of the protein and the size of the reaction which it gives; the smallest reaction which is called positive measures .5 x .5 centimeter in diameter and reactions less than this are called ± and are interpreted as questionable or slight. It is noted that after treatment the high dilutions fail to give positive reactions.

The following patient illustrates a false type of skin reaction:

Case 29. — P. J. F., tested with the wheat proteins:

Gliadin,	1–100 = (2+)	1–1,000 = (1+)	1–10,000 = (2+)	1–100,000 = (1+)
Leucosin,	1–100 = (2+)	1–1,000 = (1+)	1–10,000 = (2+)	1–100,000 = (2+)
Glutenin,	1–100 = (2+)	1–1,000 = (2+)	1–10,000 = (2+)	1–100,000 = (1+)
Globulin,	1–100 = (1+)	1–1,000 = (1+)	1–10,000 = (2+)	1–100,000 = (2+)

In this table it is noted that the size of the reaction does not diminish as the dilution of the protein increases and, furthermore, the size of the reaction varies irrespective of the dilution of the protein, and the highest dilution in some instances gave a more positive reaction than did some of the lower dilutions. This type of reaction was met with only once in the first one hundred patients who were tested. The methods employed in making the proteins which were used in the skin tests and for treatment have been described in Studies III. to IX. inclusive.

In this paper the serum complement fixation and agglutination reactions will only occasionally be mentioned since these tests were described in Studies XII. and XIII. However, in this paper the initials of each patient are given and the same initials are used throughout all the series of papers, so that a patient may be followed throughout the whole series, and by consulting each paper a complete story of the case may be put together.

The patients who gave positive skin reactions with high dilutions of a protein were first treated with the dilution next higher than that to which the patient was sensitive, and after several subcutaneous injections with this dilution the next lower dilution was used and so on until concentrated solutions were used. When treatment was given with S. pyogenes aureus and albus vaccines all patients were given the same stock vaccine of these organisms. The same holds true for those patients who were treated with a stock diphtheroid vaccine. In a few instances an autogenous vaccine was made by planting the patient's sputum on plain agar, which would rule out such types of organisms as the

influenza bacillus, the pneumococcus, and virulent streptococci. Furthermore, autogenous vaccines were not made when the predominating organism was a staphylococcus, a micrococcus, or a definite bacillus. Autogenous sputum vaccines were used only when the predominating organism was a diphtheroid, and although the type of organism was not carefully studied in each case it usually resembled a type of diphtheroid which has been described in Study II. The vaccines were killed by heating at 56° C. for two hours. Only the thick globules of sputum which were collected after a paroxysm of coughing were used for isolating the organism. Frequently several large bottles of agar were inoculated with different clumps of the same sputum and usually each bottle grew the same type of organism in approximately the same proportion of colonies, thus showing a practically homogeneous distribution of the same type of organism in the sputum.

Group I.

Patients Sensitive to and Treated with Horse Dandruff Proteins.

Case 1. — D. G., a maid aged 22, was first seen on Sept. 8, 1916.

The patient began to have asthma one year ago. The first attack was two weeks after an injection of antitoxin. At first she had asthma nearly every night, but shortly the attacks became spasmodic and she was never free from asthma for more than a month. Since the onset of asthma, the patient has taken cold easily, has had a cough all of the time and has much expectoration. The patient has lost thirty pounds in weight, is very weak, cannot dance, walk upstairs or on a level for any distance without having asthma. She cannot lie down without wheezing. At times she has raised bloody sputum and was thought to have tuberculosis, but repeated examinations of her sputum have failed to reveal tubercle bacilli. She has asthma when riding behind horses, but previous to the injection of antitoxin horses gave her no trouble.

Cutaneous reactions were positive with an alcoholic extract of horse dandruff; with the horse dandruff coagulated protein the skin reaction was positive in a dilution no higher than 1–100, with the peptone it was negative in a dilution of 1–100 and with the alkali meta-protein it was slightly positive in a dilution of 1–100,000. Horse serum protein gave a negative reaction.

Treatment was begun on Sept. 8, 1916, with two minims [1] of a dilution of 1–1,000,000 of horse dandruff alkali meta-protein subcutaneously. The following week five minims were given and after this the patient was able

to lie down without wheezing. A week later eight minims were given and following this treatment the patient had a bad cold and asthma for two days. For the next three weeks treatment was continued as before. The patient was now free from wheezing, had less cough and less expectoration; the last week was the best she had had for a year. On October 27th the dilution was changed to 1–100,000 and of this two minims were given. This was followed by less cough and the patient was now able to walk up hill without wheezing. The next week the patient was given subcutaneously four minims of the alkali meta-protein in a dilution of 1–100,000. A few hours later the patient had a bad cough, asthma, and had to sit up in bed and gasp for breath. The arm was very sore. It is noted that the last treatment was double the amount of protein which was given the previous time and evidently this sudden increase caused the bad attack of asthma; as further evidence of this the attack came on a few hours after the treatment. On November 10th the second previous dose, namely two minims of the protein in a dilution of 1–100,000, was given. On November 17th the amount of protein was increased to three minims and a week later it was increased to four minims. No inconvenience was experienced following four minims this time and even the arm was not sore, so that by gradually increasing the dose of protein no trouble resulted, but following a doubling of the previous dose a severe reaction resulted. At this time the patient was feeling stronger and was able to walk to the street car two miles distant. The cutaneous reaction had diminished a hundred-fold, it was now slightly positive with alkali meta-protein in a dilution of 1–1,000. From this time up to December 29th (a period of five weeks) five treatments were given subcutaneously with alkali meta-protein in a dilution of 1–100,000; each injection was increased one or two minims, until the last one was eleven minims. The patient now was able to dance, run, walk three miles, and sing without cough or wheezing. On Jan. 5, 1917, the dilution of protein was changed to 1–10,000 and of this two minims were injected. From this time on until February 23d (a period of seven weeks) seven treatments were given with horse dandruff alkali meta-protein in a dilution of 1–10,000, each time the amount of dilution was increased until the last injection was eight minims. The patient has felt well, has gradually increased in weight until she now weighs more than ever before, and has gained thirty-two pounds since she was first seen. At present she has a head cold, which is the first one since treatment was begun. Three weeks ago the patient rode for two hours behind a horse without having asthma; even her eyes did not water.

Summary. — This patient showed marked improvement during subcutaneous injections with horse dandruff alkali meta-protein. She gained in weight and strength, was free from colds for over four months, her cough and profuse expectoration cleared up, she became able to walk long

distances without dyspnea and was able to ride behind horses without the slightest trouble. She was relieved of asthma. This case also illustrates the importance of slowly increasing the desensitizing dose of protein. While the amounts of protein were slowly increased the patient had no trouble and improvement was steady; however, when a sudden increase in the amount of protein was given (as occurred on November 3d) asthma followed a few hours later. This patient is interesting in that horse asthma followed an injection of antitoxin, although the patient was not sensitive to horse serum, and previous to the injection of antitoxin the patient experienced no trouble from exposure to horses. The cutaneous reaction in this case would seem to show a marked difference in the three proteins which are present in horse dandruff. The peptone was negative in a dilution of 1–100, the coagulated protein was positive only in this dilution, but the alkali meta-protein was positive in a dilution of 1–100,000. Treatment with the alkali meta-protein diminished the positiveness of the skin reaction with this protein a hundred-fold.

Case 2. — S. H. G., the wife of a hostler, aged 42, was admitted to the hospital on Oct. 26, 1916, medical number 5519. A diagnosis of diabetes mellitus and bronchial asthma was made.

Family history: The patient's father, maternal grandfather, and a second cousin on her mother's side had asthma and the patient's daughter had asthma from the age of two until the age of seven, when she had whooping-cough and no asthma since then.

Present illness: Patient has had asthma for sixteen years; the first attack followed the grippe. Each attack of asthma is usually of two to three weeks' duration and some attacks are of five weeks' duration. The interval between attacks varies between a few days and a week. She has more asthma in winter than in summer, takes cold easily and has much bronchitis with asthma. She associates asthma with horses, colds, and dust. The patient has to inhale the fumes from an asthma powder every night, requires three to four pillows, sleeps poorly and is very nervous. In order to relieve the paroxysms of asthma the patient's physician has to inject morphia. The patient has had eczema for the past two years.

Cutaneous reactions were positive with the protein of S. pyogenes aureus and albus and with the alcoholic extracts of horse dandruff and cat hair. Horse dandruff alkali meta-protein and coagulated protein gave positive cutaneous reactions in a dilution of 1–1,000 and slightly positive in dilutions of 1–10,000; the peptone gave a positive reaction in a

dilution of 1–10,000. Cat hair protein gave a slightly positive cutaneous reaction in a dilution of 1–10,000 and the peptone was positive only in a dilution of 1–100. Horse and cat sera were both negative.

Treatment: Disposal of the family cat ruled out this source as a future cause. Since the patient was the wife of a hostler it seemed best to desensitize the patient with horse dandruff proteins, and the peptone to which the patient gave the strongest skin reaction was selected. On October 30th the patient was given a subcutaneous injection of four minims of horse dandruff peptóne in a dilution of 1–100,000, a week later eight minims were given, and a week later still three minims of a dilution of 1–10,000 were given. Following this last injection the patient had a sore arm, so that this same dose was repeated the next week. At this time the patient was discharged from the hospital. On November 21st, a few days after getting home, the patient woke up one morning with asthma. Two days later the patient was given subcutaneously three minims of horse dandruff peptone in a dilution of 1–10,000. During the following week the patient had a cold, bronchitis, and asthma, which was so severe that her physician injected morphia. The patient was again treated with three minims of the peptone in a dilution of 1–10,000, and except for a cold the patient had a good week. A week later four minims of the peptone were given. During the next twelve days the patient had asthma steadily for a week. Since the patient had had as frequent attacks of asthma and as many colds during this treatment as she had had previous to it, it was considered best to give S. pyogenes aureus vaccine in conjunction with the horse dandruff peptone as the patient was also sensitive to the protein of this organism. On December 19th the patient was given subcutaneously three minims of horse dandruff peptone in a dilution of 1–10,000 and 150 million of stock S. pyogenes aureus vaccine; on December 27th the dose was increased to four minims of the peptone dilution and to 200 million of the vaccine. The week following this treatment was the best the patient had had for years; she slept well, was able to lie flat in bed, and did not have to burn her asthma powder. On Jan. 3, 1917, she was given subcutaneously five minims of the horse dandruff peptone in a dilution of 1–10,000 and 200 million of the S. pyogenes aureus vaccine. A week later six minims of the peptone dilution and 250 million of the vaccine were given; during the following week she had a mild cold, but no asthma. From January 17th to January 31st the patient had grippe and stomach trouble, but no asthma. Her physician said that this was the first time the patient had been sick without also having asthma. From January 31st to February 21st five treatments were given, each time the amount of peptone dilution and vaccine was increased until the last injection consisted of ten minims of the peptone in a dilution of 1–10,000 and 450 million of S. pyogenes aureus vaccine.

Summary. — In this case the skin tests revealed four possible causes of asthma, namely S. pyogenes aureus and

albus, horse dandruff, and cat hair. The cat was disposed of. During treatment with horse dandruff peptone, the patient was only slightly improved. After treatment was begun with S. pyogenes aureus vaccine in conjunction with horse dandruff peptone, improvement was rapid and marked; the patient was free from asthma for eight weeks, even though she had a two weeks' illness which had never occurred before without asthma as a complication, she was able to dispense with her asthma powder and extra pillows, her sleep was not interrupted, and she had only one cold. This patient, therefore, illustrates multiple sensitization and the importance of treatment with more than one protein. The case also illustrates the specificity of proteins in the treatment of asthma.

Case 3. — L. T., a schoolboy aged 13, was first seen on Oct. 16, 1916.

Family history: Maternal grandmother had asthma.

Present illness: The patient has had bronchial asthma ever since birth, so his mother states. He has asthma about every three weeks throughout the warm months, and he has it oftener during the winter. Each attack is usually of three days' duration and it is accompanied by much cough, but no expectoration, and he often has vomiting with the asthma. He is unable to run without asthma. He takes cold easily, has frequent head colds, and sneezes with slight sudden changes in the temperature.

Cutaneous reactions were positive with an alcoholic extract of horse dandruff; with horse dandruff peptone and protein the skin reactions were positive in a dilution of 1–10,000 each and with the coagulated protein the reactions were slightly positive in a dilution of 1–100,000. Horse serum protein gave a negative skin reaction.

Treatment was given on October 20th, 27th, and November 3d, with two, four, and six minims of horse dandruff coagulated protein in a dilution of 1–1,000,000. On November 10th the patient rode behind a horse and patted the animal several times. The next day the patient had an attack of asthma which continued for over a week. On November 23d the dilution of the coagulated protein was increased to 1–100,000, and of this two minims were given subcutaneously. The following week the patient had asthma for two days. On December 1st the previous dose was repeated and the following week the patient had a little wheezing with exertion on four days. From December 8th to January 13th inclusive the patient was given gradually increasing amounts of the coagulated protein in a dilution of 1–100,000; the last injection consisted of nine minims. During this interval the patient had only an occasional slight attack of wheezing on exertion. At this time the patient has a better

appetite, sleeps without wheezing, has stopped sneezing with sudden changes in the temperature, and has had no head colds. At school he is running the 440 and 220-yard dashes with no more dyspnea than the other boys have. The cutaneous reaction is slightly positive with the coagulated protein in a dilution of 1–10,000, therefore the positiveness of this reaction has decreased ten times. From January 20th to February 3d inclusive treatment was continued with a 1–100,000 dilution of the coagulated protein, the last dose was fifteen minims. On February 10th the dilution was increased to 1–10,000 and of this three minims were given subcutaneously. The week following this treatment the patient had a cold in his head and on his chest, and he had dyspnea with much cough and expectoration. On February 17th the patient was feeling well and the previous dose was repeated. The next week he was given four minims of this dilution of the coagulated protein. On March 3d the cutaneous reactions were positive with the protein in a dilution of 1–100, with the peptone in a dilution of 1–1,000 and with the coagulated protein in a dilution of 1–100, but all were negative with higher dilutions, therefore these reactions had greatly decreased.

Summary. — Treatment was begun in this case with the coagulated protein in a dilution of 1–1,000,000, which was probably needlessly too low since the patient was only slightly sensitive to this protein in a dilution of 1–100,000, and since the treatment was not sufficient to protect him from asthma following exposure to horses. After treatment was begun with a dilution of 1–100,000 the patient steadily improved until he could sleep and run dashes without wheezing, was free from colds and sneezing. Thus this patient went three winter months without asthma or any other trouble. The very early age of onset of horse asthma in this patient is interesting.

Case 4. — J. T. S., a teamster aged 40, was first seen on July 28, 1916. The patient has had asthma for five years. For a few years previous to the onset of asthma he was troubled with much sneezing and cough. Each attack of asthma lasts for a day, but he sneezes and coughs all the time. All symptoms are worse in warm weather and he has little asthma in winter. The patient has noticed that all symptoms are much worse when he grooms his horses, and in warm weather he has to tie a cloth over his nose and mouth. He has indigestion after eating boiled dinners, cabbage, etc.

Cutaneous reactions were positive with an alcoholic extract of horse dandruff and negative with horse serum protein.

Treatment in this case was begun with normal horse serum, since horse dandruff had not been split up into its protein at this time. The patient stopped grooming his horses, but he continued to drive them. Following a subcutaneous injection of two minims of horse serum diluted 1–100 with saline, the patient had less sneezing. On August 2d five minims of a 1–10 dilution of horse serum were given subcutaneously; the patient's arm was very sore and he coughed and wheezed badly for two nights following this treatment. On August 9th the above treatment was repeated and again he had a lot of wheezing, cough, and expectoration. He had no trouble driving his horses, but when he attempted to groom them he sneezed, wheezed, coughed, and raised much sputum. On August 16th the patient gave positive skin reactions with horse dandruff peptone and coagulated protein, each in a dilution of 1–100. The patient was treated with eight minims of a 1–10 dilution of horse serum. During the following week the patient had asthma every time he groomed his horses. On August 23d twelve minims of a 1–10 dilution of horse serum were given subcutaneously, and following this the patient was still unable to groom his horses. On September 6th the patient was given subcutaneously five minims of horse dandruff peptone in a dilution of 1–1,000. On September 13th eight minims were given subcutaneously. The week following this treatment the patient groomed his horses every day and had no sneezing, no cough, and no asthma. On September 20th the dilution of the peptone was increased to 1–100, and of this two minims were given subcutaneously. For the next five weeks the patient was given gradually increasing amounts of this dilution of the peptone, and during this time the patient was entirely free from cough and asthma, even when he groomed his horses, and only occasionally did he sneeze at this time. The skin reactions became negative with the horse dandruff proteins in concentrated powder.

Summary. — Treatment, in this case, with horse serum in a dilution of 1–100 and 1–10 was followed by some improvement; the patient was able to drive his horses without much trouble, but he could not groom them without sneezing, coughing, and having asthma. Following the second treatment with horse dandruff peptone, however, the patient was able to groom his horses every day without cough or asthma, and only rarely did he sneeze. The cutaneous reactions, which were positive with horse dandruff peptone and coagulated protein each in a dilution of 1–100 previous to treatment with horse dandruff peptone, later became entirely negative with these proteins in a concentrated form following treatment with the peptone.

Case 5. — H. M., a schoolboy aged 10, was first seen Aug. 21, 1916. The patient has had asthma since he was three years old. The first attack followed pneumonia and bronchitis. He has had an attack of asthma about every three or four weeks and in damp weather the attacks are more frequent. Each attack lasts from two to three days. Patient has frequent colds and a cough all of the time. Four years ago following removal of his adenoids he was free from asthma for five months.

Cutaneous reactions were positive with alcoholic extracts of horse dandruff and dog hair. Horse dandruff peptone was positive in a dilution of 1–100 and dog hair peptone was positive in a dilution of 1–1,000; the other proteins in horse dandruff and dog hair were negative. The proteins of S. pyogenes aureus and albus and of horse serum gave positive skin reaction.

Treatment: The patient was treated at weekly intervals from August 24th to October 16th with stock S. pyogenes aureus vaccine in gradually increasing amounts varying from 100 million to 400 million. During these treatments the patient was free from asthma for seven weeks, although he had had two colds during this time. The week of October 16th the patient had a bad cold and an attack of asthma which lasted three days. The next week he had another attack of asthma. From the patient's mother it was learned that the patient had ridden all day on a horse team previous to one of these attacks of asthma and previous to the other attack he had played with a dog all of one day. Therefore treatment was begun with horse dandruff peptone to which the patient was sensitive. On October 26th the patient was given subcutaneously three minims of horse dandruff peptone in a dilution of 1–1,000, on November 8th five minims, and on November 15th seven minims. The patient was not seen again until Feb. 20, 1917, or fourteen weeks later. During this time (he had had no asthma until five days previous when he rode on a horse team) he had been free from cold and cough, had gained seven pounds in weight, and . . . six weeks previous to this last attack the patient had a little cough for a few days after riding on a horse team. Treatment was begun again with horse dandruff peptone.

Summary. — Since this patient gave a positive skin reaction with the protein of S. pyogenes aureus, he was given vaccines of this organism. During this treatment he was free from asthma twice as long as he had usually been previous to treatment. Since an attack of asthma followed exposure to horses, he was then given treatment with horse dandruff peptone to which he was sensitive. Although only three of these treatments were given, he remained free from asthma for thirteen weeks, and during this time he had been free from colds, had gained in weight, and had felt like a new

person. It is of interest to note that seven weeks after the last treatment, the patient had some cough after riding behind horses, but no asthma, and that six weeks later still he had asthma after riding behind horses. In other words, at the end of seven weeks he was still sufficiently desensitized to protect him from asthma from horses although he did have some cough. At the end of another period of six weeks, however, sensitization had returned to such an extent that he now had asthma from horses. This case well represents multiple sensitization and illustrates the necessity of multiple treatment with proteins in some cases.

Case 6. — C. F. J., an electrician, aged 28, has been admitted to the wards three times: on Oct. 5, 1915, medical number 3424; on Jan. 10, 1916, medical number 3934; and on Jan. 15, 1916, medical number 3971. On each admission a diagnosis of bronchial asthma was made.

Family history: A sister of the patient's mother has asthma.

Present illness: The patient has had asthma every winter since he was three years of age. Each attack is usually from ten days to two weeks' duration, and up to the age of twenty he averaged an attack every six weeks. Between the ages of twenty and twenty-two the patient was practically free from asthma; at this time he was living in the South and in the West. While he lived in the West he drove and groomed horses and was troubled with much sneezing, coughing, and expectoration; he had one attack of asthma there. Since then he has had asthma when he was exposed to horses and for the past four winters he has had three attacks of asthma each winter. He has had much sneezing and a bad cough with much expectoration during the past four winters. Besides exposure to horses, overeating, wind, and dust also cause asthma.

Cutaneous reactions on January 12th were positive with alcoholic extracts of horse dandruff and cat hair and with cat serum protein. Horse serum protein gave a negative skin reaction. On October 24th cutaneous reactions were positive with horse dandruff alkali meta-protein and coagulated protein in dilutions of 1–1,000 and with the peptone in a dilution of 1–10,000. Cat hair alkali meta-protein gave a positive reaction in a dilution of 1–1,000, but the peptone was negative in concentrated amounts as was also the acid meta-protein. Dog hair alkali meta-protein gave a positive skin reaction in a dilution of 1–1,000, but the peptone was negative in a dilution of 1–100. On December 12th cutaneous reactions were positive with wheat proteins; glutenin, leucosin, and natural proteose gave slightly positive reactions in dilutions of 1–1,000 and globulin in a dilution of 1–10,000, but gliadin and artificial proteose gave negative reactions in concentrated powder.

Treatment: During July the patient was given four subcutaneous injections with a dilution of 1–100 of horse serum in amounts of two, two, three, and four minims respectively. Following these treatments the patient spent two weeks on a farm without any trouble. A month later he spent a week end in the country and on retiring in a closed room he had asthma, which left him in a few minutes on going out of doors. He returned to this room later and again had asthma, which quickly left him on going out of doors. He retired in another room without trouble. The next morning on investigating the room in which he had had asthma the previous night, he found a lot of dried catnip under the bed; on smelling of this he immediately choked up. An extract was made of a little of this catnip and a skin test was done: although it did not give a raised reaction the surrounding skin was red. On another occasion the patient wheezed after playing with a cat. Beginning on October 24th the patient was given a few weekly treatments with horse dandruff peptone in a dilution of 1–100,000. On Thanksgiving night the patient had a bad attack of asthma and vomiting. Following this, wheat was omitted from the patient's diet and treatment was begun with wheat globulin in conjunction with horse dandruff peptone. From December 12th to January 23d seven subcutaneous injections were given with horse dandruff peptone and wheat globulin, each in a dilution of 1–100,000; the first injection was with three minims of each and the last was with nine minims of each. From February 1st to February 28th this treatment was continued with a dilution of 1–10,000 of each protein. On February 28th the cutaneous reactions were positive with all horse dandruff proteins in a dilution of 1–100, but they were negative with higher dilutions; cat hair protein was still positive in a dilution of 1–1,000 as before; wheat leucosin and glutinin were slightly positive in a dilution of 1–100 and globulin was a good positive in a dilution of 1–100. The patient has been free from colds, cough, expectoration, wheezing, and asthma; the past Christmas was the first one that he has been free from asthma.

Summary.—This case well illustrates the wide source of proteins to which a patient may be sensitive. He was sensitive to horse dandruff proteins and had asthma when exposed to horses; he was sensitive to cat hair proteins and had asthma when in immediate contact with cats; he was sensitive to wheat proteins and had asthma when he overate; and always had asthma following Thanksgiving and Christmas dinners; inhalation of the dust from dry catnip caused him to have asthma on two occasions. Since the patient was so sensitive to wheat proteins one would think that he would have more asthma than he did. But as he is a strong, hardworking man it is probable that his digestion for these

proteins is usually complete and none escapes into the circulation undigested; but following excessive eating, as occurs at the holiday period in conjunction with the sedentary habits at such times, some undigested wheat protein does escape into the circulation and causes asthma. This explanation is borne out by the fact that this past Christmas, while he was on a wheat-free diet, was the first one that he was free from asthma. During treatment with horse dandruff peptone and wheat globulin the patient was free from asthma, and from his usual colds, cough, and expectoration. It is interesting to note that treatment with one of the proteins in horse dandruff decreased his sensitiveness to all of the horse dandruff proteins tenfold or more, and the same is true in regard to the wheat proteins; but his sensitiveness to cat hair proteins was not changed. From these latter facts it would seem that the horse dandruff proteins were closely related to each other, and that the wheat proteins also were closely related to each other, but that there is no close relationship between the proteins in horse dandruff and those in cat hair. That catnip should cause asthma in this case illustrates the unlimited possibilities of the complications in the successful treatment of bronchial asthma. Following all of this treatment the skin reactions had greatly diminished; they were now positive with the horse dandruff proteins in a dilution of 1–100 only and with the wheat proteins they were slightly positive in this dilution. The proteins of cat and dog hair remained unchanged.

Case 7. — R. H. W., a schoolboy aged 11, was first seen on Aug. 12, 1916.

Family history: The patient's father has wheezed for years.

Present illness: The patient began to have asthma five years ago following whooping-cough. In the winter he has no real spasmodic asthma, but he wheezes with colds and he has three or four colds each winter. During the summer months the patient has attacks of spasmodic asthma, each attack is from twenty to thirty minutes' duration and is accompanied by an unproductive cough. The amount of asthma varies each summer; last summer the patient had little asthma, but this summer he has had it most of the time and has been so bad that he could not stay in the country. Asthma appears immediately after riding behind horses.

Cutaneous reactions were positive with an alcoholic extract of horse dandruff and with the pollen of red top and timothy. The peptone and the alcohol soluble protein of horse dandruff gave negative skin reactions, but the coagulated protein and the alkali meta-protein gave slightly positive skin reactions, each in a dilution of 1–10,000.

Treatment was begun on August 18th with the alkali meta-protein in a dilution of 1–100,000 and of this five minims were given subcutaneously. During the next three weeks three treatments were given with a 1–10,000 dilution of this protein, two, five, and eight minims respectively. On September 6th the patient returned to the country.

Results. — Following four treatments with horse dandruff alkali meta-protein the patient returned to the country and had no asthma there. During this winter the patient has been free from the usual winter colds and wheezing and he has ridden behind horses without trouble. He has put on ten pounds in weight and has been able to do as other boys without the consequences which used to follow such exposure.

Case 8. — C. S., a schoolgirl aged 10, was first seen on Jan. 5, 1917. The patient has had asthma spasmodically since two years of age. The attacks consist of a whistling in the chest and coughing for four or five days and a part of this time she has to sit up in bed for breath. Asthma follows exposure to horses, hay dust, street dust, cold air, and running against the wind. Attacks have gradually become less frequent and less severe; they usually occur now only after riding behind a horse and they last a day and a half. The patient wheezes with a cold, and is short of breath in cold air, when running against the wind, and occasionally at night.

Cutaneous reactions were positive with an alcoholic extract of horse dandruff and with the pollen of timothy and rag weed. Horse dandruff protein and peptone were positive in dilutions of 1–10,000, but the coagulated protein was negative in this dilution. Horse serum protein gave a negative reaction.

Treatment was given subcutaneously with a mixture of the peptone and protein in dilutions of 1–100,000 from January 5th to February 9th inclusive; the first dose was two minims and the last one was ten minims. From this time on treatment was continued with a mixture of these two proteins in a dilution of 1–10,000.

Results. — The patient says that she can run in the wind now without trouble, that she has been free from colds and has not wheezed at all this winter.

Case 9. — F. L. D., a business man aged 48, was first seen on Dec. 28, 1916.

Asthma began twenty-five years ago with a sudden onset one afternoon while riding horseback. Asthma occurs only after contact with horses and sometimes he can ride horseback without trouble. The attacks begin with itching of the eyes and running of the nose while exposed to horses, and a few hours after leaving the horse he has dyspnea, followed by asthma. Rarely is the asthma so severe that he has to sit up all night. The attacks are milder in winter and are most severe in damp weather. Contact with other animals causes the patient no trouble and a dog has slept on the bed with the patient for twenty-five years.

Cutaneous reactions were positive with alcoholic extracts of horse dandruff, wool, cat, dog, and cattle hair. Horse dandruff protein, peptone, and coagulated protein all gave positive skin reactions in a dilution of 1–100,000. The protein and peptone from cat and dog hair were negative in dilutions of 1–1,000. The sera of each of these animals gave negative skin reactions.

Treatment was begun on January 3d with three minims of a mixture of all three proteins from horse dandruff in a dilution of 1–1,000,000. On January 10th six minims were given. On January 16th by mistake eight minims of each protein in a dilution of 1–1,000,000 were given subcutaneously; thus twenty-four minims, which was four times the previous dose, and which was the equivalent of two and a half minims of a 1–100.000 dilution, were given. This sudden and greatly increased dose of protein gave the patient no inconvenience except for a sore arm. Treatment has been continued with a dilution of 1–100,000 of a mixture of the three proteins, but at present no opportunity has presented itself for testing the patient's improvement.

This case is presented in order to bring out the following interesting facts: The sudden onset of symptoms. The extreme sensitiveness of the patient's skin to the proteins in horse dandruff in contrast to the slight sensitiveness to the proteins in cat and dog hair, although the patient has been more exposed to the dog than to the horse.

Group II.

Patients Sensitive to and Treated with Cat Hair Proteins.

Case 10. — G. V. Y., a colored waitress aged 28, was admitted to the hospital on July 13, 1916, medical number 4958. A diagnosis of bronchial asthma, chronic bronchitis, and emphysema was made.

The patient has had asthma since December, 1910. The first attack began with a cold and continued for four days. For the first six months attacks occurred once a week, later on she had one every two or three weeks, and for the past two years they have come at less frequent intervals, but

are more severe; she is never free from asthma for more than a month. Occasionally an attack would continue two weeks, but the usual duration was several days. Attacks follow exposure to east winds and sometimes they follow eating baked beans. The patient usually vomits and belches gas during an attack. The patient feels wheezed up when near cats, and where she now works there are ten of them.

Cutaneous reactions were positive with an alcoholic extract of cat hair; with the peptone and the alkali meta-protein of cat hair they were positive in a dilution of 1–1,000,000, with the concentrated acid meta-protein they were negative. The powdered protein of cat serum gave a negative reaction.

Treatment: On July 27th the patient was given subcutaneously two minims of cat hair alkali meta-protein in a dilution of 1–10,000,000, and a week later two minims of the same in a dilution of 1–1,000,000. On August 9th following two minims of the same in a dilution of 1–100,000 the patient began to have severe asthma two hours after the injection and the attack of asthma continued off and on for a week, although the patient was away from cats during this time. On the three following weekly treatments the patient was given subcutaneously three, five, and six minims respectively of cat hair alkali meta-protein in a dilution of 1–1,000,000 without any trouble. A week later, on September 13th, the patient was given subcutaneously, as an experiment, eight minims of horse dandruff alkali meta-protein in a dilution of 1–1,000; this was followed by more or less of a sore arm, but no other trouble resulted. The next two treatments consisted of eight and ten minims of cat hair alkali meta-protein in a dilution of 1–1,000,000. On October 4th, since the patient had been free from wheezing and asthma for five weeks, the dilution of cat hair protein was increased to 1–100,000, of which two minims were given without any reaction. Throughout October, November, and December eleven treatments were given with cat hair alkali meta-protein in a dilution of 1–100,000; each succeeding dose was increased by one or two minims until the last one on January 3d was eleven minims. During this time the patient had no wheezing or asthma except for one day. On November 22d cutaneous reactions were repeated with cat hair alkali meta-protein and peptone, and both were negative in dilutions of 1–100,000. During January and February the patient was treated subcutaneously with cat hair alkali meta-protein in a dilution of 1–10,000 and had wheezing on one day. On February 7th cutaneous reactions were again repeated with cat hair proteins and the peptone was still positive in a dilution of 1–10,000, but the alkali meta-protein was only questionably positive in this dilution.

Summary. — During twenty-seven consecutive weeks of treatment the patient had asthma on only two days, thus showing remarkable improvement and freedom from asthma, even though there were several cats in the house where she

worked and she had to feed them, and has made a pet of one of them. The sensitiveness of the patient, as demonstrated by the cutaneous reaction, to cat hair is remarkable since both proteins in cat hair gave a positive reaction in a dilution of 1–1,000,000. This extreme sensitiveness of the patient is further borne out by treatment. Two minims of cat hair alkali meta-protein in a dilution of 1–1,000,000 were injected subcutaneously without any trouble, but when two minims of this protein in a dilution of 1–100,000 were injected a severe attack of asthma began two hours later. It is interesting that this attack of asthma should continue for a week following one exposure to the antigen. After a few doses of the protein in a dilution of 1–1,000,000, however, the patient was sufficiently desensitized to withstand a dilution of 1–100,000 which had previously caused severe asthma. During treatment the positiveness of the cutaneous reaction decreased a hundred-fold. Although treatment was given with only the alkali meta-protein, the cutaneous reaction decreased with the peptone as well as with the protein, so that both the peptone and the protein would seem to be closely related. In conjunction with this it is interesting to note that a treatment with horse dandruff alkali meta-protein in a dilution of 1–1,000 caused no trouble other than a local soreness, so that there would seem to be no relationship between the alkali meta-protein from cat hair and that from horse dandruff. The cutaneous reaction also bears this out since the patient gave a negative reaction with horse dandruff proteins in a concentrated form.

Case 11. — M. D., a woman aged 38, was admitted to the medical ward on Nov. 22, 1916, medical number 5694. A diagnosis of bronchial asthma was made.

The patient had her first attack of asthma ten years ago during a nervous strain. The next attack was six months later during menstruation. From this time on she has had asthma with her periods, which became very scanty in the flow, and the attacks of asthma became more frequent until she had an attack nearly every day. Then attacks became more and more infrequent until she had asthma only with menstruation. Last spring while in a run-down condition she again had asthma almost every

day. Since October she has had asthma almost every day, but she has it worse with her periods. She has had a bad cough for some time, and dust and exertion cause asthma; she wheezes most of the time. She has a cat at home, but will not part with it.

Cutaneous reactions were positive with the three horse dandruff proteins, each in a dilution of 1–10,000; with cat hair proteins in a dilution of 1–100,000, with dog hair proteins in a dilution of 1–100, and with the pollen of rag weed. The serum proteins of these animals were negative.

Treatment: The patient was given at weekly intervals seven subcutaneous injections of cat hair alkali meta-protein in a dilution of 1–1,000,000; the amounts were gradually increased from two to eleven minims. During this time she had asthma for one day every week and wheezed some at other times; she had a bad cough. During the next eight weeks treatment was given with the cat hair protein in a dilution of 1–1,000,000 and in addition she was given a vaccine with a diphtheroid organism from her sputum; the amount of each was gradually increased each week. During this time the patient has been free from asthma, wheezing, and cough since the first week. She claims that she has not felt so well for ten years and she has gained six pounds in weight. She is no longer nervous and irritable as she was previously.

Summary. — It is difficult to tell whether it was the treatment with the more concentrated solution of cat hair protein or the sputum vaccine or both that relieved the patient of all symptoms.

Case 12. — P. D., a schoolboy aged 11, was first seen on Oct. 31, 1916.

Family history: The patient's father has had asthma for 25 years and a paternal uncle had asthma.

Present illness: At the age of five the patient had diphtheria, scarlet fever, pneumonia, and pleurisy, all inside of eleven weeks. Two years later when the patient was seven years old he began to have asthma. The first attack began with a cold, shortness of breath, and choking spells. During the first two years he had four or five attacks of asthma only in the winter time. During the next winter he had ten attacks and this fall he has already had two attacks. Each attack varies between three and ten days' duration and is usually accompanied by vomiting.

Cutaneous reactions were positive with alcoholic extracts of horse dandruff, cat and dog hair, and with the serum proteins of these animals. Horse dandruff alkali meta-protein and peptone were slightly positive in a dilution of 1–1,000 and coagulated protein was positive in a dilution of 1–100; cat hair peptone was positive in a dilution of 1–1,000, but the alkali meta-protein was negative with this dilution; dog hair alkali meta-protein was slightly positive in a dilution of 1–1,000, but the peptone was negative in a dilution of 1–100. The natural and artificial proteoses were both slightly positive, but the other wheat proteins were negative.

Treatment: The patient was given subcutaneously five injections of cat hair peptone in a dilution of 1–10,000, since he reacted most strongly to this form of protein; two, three, four, five, and six minims were given respectively. In addition to this treatment the patient was on a wheat-free diet for two weeks. During this treatment the patient had asthma a part of each week, some weeks he had only one day of asthma, other weeks he had several days of asthma. From December 12th to March 12th the patient was given vaccines with a diphtheroid from his own sputum, each week the amount of vaccine was increased, beginning with 200 million and the last treatment was 800 million. During this time the patient had no asthma or cough after the second vaccine treatment.

Summary. — In this case the sensitization, which was not marked to the proteins in the dandruff of the horse and in the hair of the cat and the dog, played no part at the present time in the cause of asthma, since during treatment with the protein to which he was most sensitive he continued to have attacks of asthma every week. On the assumption that the slight reactions with wheat proteoses might mean something, the patient was put on a wheat-free diet, but no improvement resulted. During the three months that he was treated with a vaccine made from the predominating organism in his sputum (which was a diphtheroid organism), the patient was free from all symptoms after the second dose. During the corresponding three months of last winter the patient lost seven weeks of school, whereas this winter he lost not a day. He has also been free from cough, expectoration, frequent colds, and attacks of vomiting — all of which he had at frequent intervals last winter.

Group III.

Patients Sensitive to and Treated with the Proteins in Wheat.

Case 13. — D. A. G., a boy aged 19, was first seen on Oct. 16, 1916.

Family history: The patient's maternal grandfather had asthma.

Present illness: At six weeks of age the patient began to have eczema; at three months of age, bronchitis, and at three years of age he had asthma. He has had all three of these diseases ever since. Between the ages of twelve and fourteen the patient was in bed days at a time with asthma, but since then the attacks of asthma have been from one to three days' duration, although he wheezes every day. The patient associates asthma with changes in the weather, with horses, and with colds. The patient is troubled with running of the nose all of the time and he has

hay fever and asthma every July and September. Two years ago removal of adenoids and a spur from his nose was followed by no relief. He has to burn an asthma powder every day.

Cutaneous reactions were positive with the protein of S. pyogenes aureus and albus, with an alcoholic extract of horse dandruff, and slightly positive with the protein of peas and horse serum. On October 18th cutaneous reactions were positive with the protein of S. pyogenes aureus and albus, with the pollen of rag weed and timothy, with the peptone, alkali meta-protein and coagulated protein of horse dandruff, each in a dilution of 1–10,000 and slightly positive with the alkali meta-protein and peptone of cat hair, each in a dilution of 1–1,000. On November 20th cutaneous reactions were positive with the wheat proteins glutenin and gliadin, they were 2 + with natural proteose, 3 + with globulin and slightly positive with artificial proteose and whole wheat. Leucosin gave a negative reaction and so did globulin and natural proteose in a dilution of 1–100.

Treatment: The patient was given three treatments with stock vaccines of S. pyogenes aureus and albus without benefit. Three treatments were then given subcutaneously with a mixture of the three horse dandruff proteins in a dilution of 1–100,000. During these latter treatments the patient had no spasmodic asthma. On November 20th the patient was put on a wheat-free diet and was given three subcutaneous injections at weekly intervals of three, four, and five minims, respectively, of whole wheat protein in a dilution of 1–500 in conjunction with two, three, and four minims, respectively, of a mixture of the three horse dandruff proteins in a dilution of 1–10,000. During this time the patient felt better than usual. He was free from asthma and wheezing and he no longer burned his asthma powder. From December 18th to February 19th, the patient was given nine subcutaneous injections of whole wheat protein in a dilution of 1–500; each time the amounts were gradually increased, beginning with five minims and ending with eleven minims. During this time the patient did not burn his asthma powder and he was free from asthma and wheezing with the exception of two days after eating spaghetti; the patient's eczema had gradually improved until his arms and hands were free from it.

Summary. — This patient illustrates multiple sensitization and the specificity of proteins in the treatment of asthma. Treatment with stock vaccines of S. pyogenes aureus and albus was of no benefit. Following treatment with horse dandruff proteins alone the patient had no spasmodic asthma. Treatment with horse dandruff proteins in conjunction with whole wheat protein and a wheat-free diet was followed by relief from asthma and wheezing and the patient discontinued burning his asthma powder. During the next two

months while he was on a wheat-poor diet and was being treated with whole wheat protein he was free from all symptoms, with the exception of two days when he ate an increased amount of bread and spaghetti; during the latter part of this period he had been eating a little toasted bread. The patient's eczema had disappeared from his arms and hands and it was greatly improved on other parts of his body. The lack of knowledge on the part of the patient that spaghetti was made of wheat illustrates a complication in the treatment of patients who are on a wheat-free diet.

Case 14. — C. K., a clerk aged 32, was first seen on June 12, 1916.

Asthma began eight years ago with a cold on the chest. During the first six years the patient had an attack every four of five weeks and each attack was with a cold. During the past two years the patient has had asthma every week and each attack was of one or two days' duration. During the past two months the patient has had asthma every night; she would wake up with asthma. In the morning she would cough and raise much. Thus the attacks of asthma have gradually become more and more frequent, but less severe. Examination of nose and throat was negative.

Cutaneous reactions were slightly positive with whole wheat protein and were very positive with the natural proteose of wheat, but they were negative with the remaining wheat proteins.

Treatment: The patient was kept on a wheat-free diet for seven weeks, during which time there was little improvement; she continued to wheeze about every night and still had the morning cough and sputum, but these symptoms were less marked. From August 7th to September 16th the patient was treated subcutaneously at weekly intervals with whole wheat protein in a dilution of 1–500; the first dose was three minims and the last was fourteen minims. During this time the patient had asthma on only one day, but she continued to wheeze more or less, although her cough was considerably less. From September 23d to November 11th the patient was treated subcutaneously at weekly intervals with wheat natural proteose in a dilution of 1–100. Following the first injection, which was three minims, the patient had severe asthma for three days and had to sit up all of one night. The next treatment was with one minim and succeeding treatments were with gradually increasing amounts. The patient had no more asthma, wheezing gradually decreased until she had none at all, and her cough became less and less until she had none. Treatment was discontinued on September 11th. The patient was not seen again until March 8, 1917, four months later. During this time she had had only one attack of asthma, which was on the day following Thanksgiving, she has had no wheezing, and only a slight cough at times.

Summary. — It is interesting that this patient was sensitive to only one of the proteins in wheat, namely, the natural proteose. This is substantiated by the fact that the patient had a bad attack of asthma following an increased injection of natural proteose, that following repeated small doses the patient rapidly improved until she was free from all symptoms, and that she showed little or no improvement following repeated injections of the whole wheat protein which contained only traces of natural proteose. During the four months since treatment was discontinued the patient has been free from symptoms with the exception of one day, which was the day following Thanksgiving.

Case 15. — C. N. E., a clerk aged 25, was admitted to the medical wards on June 19, 1916, medical number 4848. A diagnosis of bronchial asthma was made.

The patient has had asthma for twenty years, since an attack of whooping-cough, and he has had a constant cough ever since. Attacks of asthma usually occur at night, but sometimes he has them during the day. The attacks are from one to three nights' duration and come at irregular intervals, but he is never free for more than a few nights. He has more asthma in winter than in summer and he has frequent head colds. He coughs and wheezes all of the time and exposure to wind, cold weather, hay dust, rag weed pollen, and horses, aggravate these symptoms and cause him to have real asthma. He gets out of breath on slight exertion. He has to burn an asthma powder every night on retiring, and often he burns it several times in the night, and occasionally during the day. Removal of adenoids and tonsils was followed by no relief from asthma, cough, or wheezing.

Cutaneous reactions on June 20th were positive with alcoholic extracts of the dandruff of the horse and the hair of the cat, dog, and cattle; the serum protein of these animals gave positive reactions The wheat proteins, natural proteose, glutenin, gliadin, and leucosin gave positive reactions, but globulin gave a negative skin reaction. The protein of peas and raw potato gave positive reactions. On June 30th cutaneous reactions were positive with cat hair alkali meta-protein in a dilution of 1–1,000, but negative with the peptone and the acid meta-protein in a concentrated form. On August 11th cutaneous reactions were positive with horse dandruff peptone and alkali meta-protein, each in a dilution of 1–10,000 and with the coagulated protein in a dilution of 1–100,000. Dog hair alkali meta-protein gave a positive reaction in a dilution no higher than 1–100, but the peptone was positive in a dilution of 1–10,000.

Treatment: Beginning on June 20th wheat, pea, and potato were excluded from the patient's diet. During seven weeks of this restricted

diet the patient thought that he gradually improved, since he had been able to do harder work and had accomplished more than usual, and in the past four weeks he had had only two real attacks of asthma, one of which followed eating beans and the other followed exposure to east wind. From August 21st to October 24th the patient was treated subcutaneously with gradually increasing amounts of horse dandruff coagulated protein, first in a dilution of 1–1,000,000, and later in a dilution of 1–100,000 in conjunction with a wheat-free diet. During this time the patient thought that he was no more free from asthma than he had been when on a wheat-free diet alone. The patient was now allowed to eat wheat for a week and he had real asthma all of this time. On November 6th the patient was put back on a wheat-free diet and treatment was begun with subcutaneous injections of wheat globulin and glutenin, each in a dilution of 1–1,000; for six weeks gradual increasing amounts of these proteins were given. During this time the patient was free from real attacks of asthma, but he continued to have more or less wheezing and cough and he burned his asthma powder on retiring. The patient's own statement was that "he did not have enough wheezing to bother him any." This treatment has been continued and the patient has continued to have no real asthma, except on two occasions when he tried the effect of eating bread. He now notices that cold air and head and chest colds do not cause him to have asthma, that he no longer has to be careful about his habits or his diet other than wheat, and he is not prevented from doing anything which he wants to for fear of asthma. He still wheezes more or less following exertion. Following this treatment with the wheat proteins the skin reactions became negative with them, but the positiveness of the reactions with the proteins of horse dandruff, cat, and dog hair was not changed.

Summary. — This patient illustrates multiple sensitization and because of it the difficulty in proper treatment. He also demonstrates the specificity of proteins in the cause and treatment of bronchial asthma.

Case 16. — F. A., a schoolgirl aged 17, was admitted to the medical ward on May 15, 1916, medical number 4666. A diagnosis of bronchial asthma was made.

Family history: The patient's grandmother had asthma and her oldest brother and a cousin have asthma.

Present illness: As a baby the patient had bronchitis and asthma at night until she was four years old. Between the ages of four and eleven she had practically no asthma. At eleven she began to have a tightness across her chest and wheezing, which gradually increased until for the past few years she has had spasmodic asthma throughout the year and wheezing between the attacks of real asthma. Attacks usually come at night and prevent sleep, but they follow exercise, exertion, overeating, and

exposure to wind in the daytime. The patient is very constipated and with spasmodic asthma she has griping in her abdomen. She burns an asthma powder every night and often she has had to use it during the day. She takes cold easily. A year ago tonsils, adenoids, a nasal growth, and spur were removed without any relief from asthma. Bismuth studies done at this hospital showed a low and hook-shaped stomach, a cecum fixed close to the pubic bone, and a low transverse colon.

Cutaneous reactions were positive with alcoholic extracts of the hair of cattle and cat, with the proteins of wheat and with the pollens of rag weed and clover; with the protein of chicken meat and oat they were slightly positive.

Treatment: The family cat was disposed of and the patient was kept on a wheat-free diet for five weeks; during this time she had only one attack of asthma and very little wheezing. From June 20th to September 11th a wheat-free diet was continued and during this time she had only three slight attacks of wheezing, all of which followed the eating of wheat in some form. During the next two months subcutaneous injections of whole wheat protein in a dilution of 1–500 were given at weekly intervals. The patient was free from symptoms until the amounts which were injected were increased to ten minims when she had asthma. A treatment with a mixture of the individual wheat proteins in dilutions of 1–1,000 was followed by severe asthma which continued for a week. Throughout December, January, and February subcutaneous injections of a mixture of the wheat proteins in dilutions of 1–10,000 were given while the patient was still on a wheat-free diet. Each dose of these proteins was kept low, since large amounts were followed by wheezing. The patient is now able to play basket ball on her college team, she has no constipation or griping in her abdomen, and cold weather, fatigue, or exertion cause no asthma. She has not burned her asthma powder for four months.

Summary. — This patient had asthma while she was eating wheat and while she was being treated subcutaneously with large doses of whole wheat protein or with large doses of the individual wheat proteins in high dilutions. The case illustrates the difficulty in desensitizing patients with the wheat proteins. The patient has been free from symptoms while on a wheat-free diet in conjunction with small doses of the wheat proteins and she has become accustomed to exposure to cold, exercise, exertion, and windy weather without having wheezing. She has given up her asthma powder entirely. Her tolerance for eating wheat, however, has evidently not increased. The abnormal findings in the patient's gastro-intestinal tract, as was revealed by bismuth studies, are of interest.

Case 17. — G. B., a girl aged 17, was first seen on Oct. 25, 1916.

Family history: The patient's mother and a brother had asthma at three years of age and the patient's maternal grandmother and a paternal uncle had asthma.

Present illness: The patient first had real spasmodic asthma at eighteen months of age and it continued for years. In recent years she has had little spasmodic asthma, but she wheezes all of the time and has bronchitis with much cough and expectoration most of the time. Bilious attacks and vomiting now accompany spasmodic asthma. Because of constant wheezing, which is more marked on exertion, the patient goes out very little and she has been prevented from going to any kind of amusement. The patient had eczema from six months to four years of age, then was free from it until the past year, when she has had it ever since. Her eyes are very irritable, the lids are inflamed, and the scleræ are injected.

Cutaneous reactions were positive with alcoholic extracts of horse dandruff, cattle hair, and wool, with the protein of S. pyogenes aureus and albus, with the protein of peas and celery, with the pollen of rag weed and goldenrod, with the wheat proteins leucosin, gliadin, glutenin, and globulin, but they were negative with the wheat proteoses and with the serum protein of the horse and of cattle. Horse dandruff alkali metaprotein and coagulated protein gave positive reactions in dilutions of 1-10,000 and the peptone in a dilution of 1-1,000.

Treatment: The patient was given subcutaneously three minims of whole wheat protein in a dilution of 1–500 and much improvement followed. The next week eight minims were given and the patient was much worse following this dose. The next week only five minims were given and she had a good week. Following another dose of seven minims the patient had no wheezing or cough and she went to the theater nine times this week. The next week the patient was given a mixture of the four wheat proteins, to which she was sensitive, in a dilution of 1–1,1000; four minims were given subcutaneously. Following this treatment the patient was very much worse and wheezed all of the time. All treatment was omitted and the patient was put on a wheat-free diet. After a week of wheat-free diet she began to gradually improve and during the third week she had no asthma, wheezing, or cough, she felt well and the eczema was improved. The following week by mistake the patient ate some wheat and had a relapse of asthma. After avoiding wheat again for a week she again began to improve until she became free of asthma and wheezing. A second time the patient ate wheat without knowing it, until after it was too late, and she had another relapse. Finally the patient went four weeks without eating wheat and during this time she was treated subcutaneously with whole wheat protein in a dilution of 1–500. The patient became free from wheezing and cough and the eczema was improved.

Summary. — Although the patient was sensitive to several types of proteins it would seem that wheat was the cause of her asthma and possibly of her eczema. The case well illustrates the difficulty in keeping any one on a wheat-free diet and the difficulty in attempting to desensitize a patient with wheat proteins. Improvement followed small doses of whole wheat, but relapses followed larger doses and she could not tolerate small doses of the pure proteins. Following four weeks of successful management the patient became free from all symptoms with the exception of eczema, which was improved.

Case 18. — G. MacD., a housewife aged 28, was first seen on Sept. 12, 1916. She was admitted to the ward on November 1st, medical number 5532. A diagnosis of chronic bronchitis, questionable bronchial asthma, and questionable heroin poisoning was made.

Asthma began two years ago with a cold and bronchitis. During the past year she has had asthma every night, and a bad cough every night and morning with profuse expectoration, which seems to relieve the asthma for a few hours. She has frequent colds and has a spur in her nose.

Cutaneous reactions were slightly positive with the wheat proteins globulin and natural proteose on repeated tests, but the other wheat proteins always gave negative reactions.

Treatment was given with her own sputum vaccine for six weeks without any improvement. During a part of this time she was in this hospital and had asthma every night, but temporary relief followed the injection of adrenalin chloride in a dilution of 1–1,000. On November 20th the patient was put on a wheat-free diet and was given subcutaneous injections of whole wheat protein at weekly intervals. During this time she had little or no wheezing and had real asthma only once when she had eaten spaghetti. She became able, after a few weeks, to eat crackers without wheezing, but when the injections of whole wheat protein got as high as ten minims she began to have some wheezing. On returning to a strict wheat-free diet she again became free of asthma. She was then sent to a sanitorium for treatment for heroin habit.

Summary. — On a wheat-free diet the patient was practically free from wheezing and cough, but following large amounts of whole wheat when it was injected subcutaneously and following the ingestion of spaghetti the patient had wheezing and cough.

Case 19. — M. S., a housewife aged 31, was admitted to the medical wards on June 2, 1916, medical number 4768; on June 20, 1916, medical number 4863; and on Aug. 25, 1916, medical number 5200. Each time a diagnosis of bronchial asthma, chronic bronchitis, and emphysema was made and on the last admission the diagnosis of pregnancy was also made.

Family history: The patient's father had asthma.

Present illness: The patient has had asthma for fifteen years and the first attack began with a cold. For the first few years the patient had an attack of asthma only two or three times a year, but the attacks gradually became more and more frequent until at present she has asthma once a week. Usually each attack lasts only one night, but the present one has continued for a week and it is more severe than usual. The patient has asthma at all times of the year and she takes cold easily winter and summer. She associates asthma with worry, bad odors, exertion, train smoke, and wind. Often she wakes up at night with asthma.

Cutaneous reactions were slightly positive with the protein of whole wheat, raw potato, and bean.

Treatment: While the patient was on a diet free from wheat and potato for ten days she had only one attack of asthma and she felt stronger and better. On June 19th the patient was discharged from the hospital. The next day she was readmitted because of a severe attack of asthma which followed a light supper consisting of bread, cake, and milk. The patient was given at weekly intervals twelve treatments with a stock vaccine of S. pyogenes aureus in gradually increasing amounts from 200 to 750 million each time. Vaccines made from this organism were given because the patient's serum agglutinated it. During this treatment there was gradual improvement, the attacks of asthma became less frequent and less severe, and during the last five weeks she had only three attacks each of a few hours' duration, although previous to this treatment she was having one or two attacks every night. On August 25th the patient was again admitted to the hospital in a severe attack of asthma. Treatment was begun with whole wheat protein in a dilution of 1–500 and later a dilution of 1–100 was given. After the first treatment the patient had no more real asthma, and the wheezing, cough, and expectoration gradually cleared and treatment was discontinued. Six weeks later the patient returned, having gone through childbirth without any trouble and having had a cold for three weeks. The previous night she had some wheezing so she came for more treatment. Treatment with whole wheat in a dilution of 1–500 was given as before for four times without complete relief, but with much improvement. Then her sputum vaccine was combined with the whole wheat treatments with no better results. After this stock S. pyogenes aureus vaccines were combined with the whole wheat, and improvement began and continued until she became free from symptoms.

Summary. — In this case it would seem that both the wheat proteins and S. pyogenes aureus played a part in the

cause of asthma, since on two occasions a combination of the two relieved all symptoms, but each alone or with other bacteria had only a partial beneficial effect.

Case 20. — W. P. H., an accountant aged 25, was admitted to the medical ward on Nov. 13, 1916, medical number 5586. A diagnosis of bronchial asthma, chronic bronchitis, emphysema, and intestinal stasis was made.

The patient has had asthma for ten years. He averages an attack every two to three weeks and attacks continue from three days to a week. Following an operation for appendicitis a year ago the attacks changed in character; they have become more frequent, more severe, and are associated with overeating and with indigestion, which consists of gas and a feeling of fulness in his stomach. During the past three months he has wheezed all of the time, although wheezing is worse at times than at others. Bismuth studies which were done at this hospital showed a low stomach, a low redundant colon, and a definite ileal stasis.

Cutaneous reactions were positive with the protein of spinach, corn, rye, whole wheat, wheat globulin, gliadin, leucosin, and glutenin; wheat proteoses were negative and barley protein was slightly positive. Wheat globulin, leucosin, and glutenin were positive in dilutions of 1–100, but negative with higher dilutions.

Treatment: The patient was put on a wheat-free diet and was given subcutaneously five minims of a mixture of all of the wheat proteins, to which he was sensitive, in a dilution of 1–1,000. This was followed by less wheezing and he no longer had that "all gone feeling" in his stomach. The next week he was given eight minims of the above mixture and three hours later he had severe asthma, which continued for three days, and his arm was very sore. The next week only five minims of the mixture was given and he had a good week with no wheezing or discomfort. The next week he was given six minims and he continued to be free from symptoms. During the following week the patient ate some wheat and he was given five minims of the mixture as previously. Following this treatment the patient had asthma for over a week. Treatment was discontinued and the patient was kept on a wheat-free diet for the next three months. During this time he had a little wheezing at night when he was overtired, but not often enough to cause any inconvenience, and he had no real asthma with the exception of the two days following Christmas Day. On March 10th, three months after treatment was discontinued, the cutaneous reactions were greatly decreased with the proteins which previous to treatment had given good positive reactions. The proteins of rye, corn, wheat, gliadin, and barley were negative; the wheat proteins, globulin, glutenin, leucosin, and proteoses, were slightly positive in concentrated amounts.

Summary. — This patient, like many others who are sensitive to wheat proteins, shows an inability to take large

amounts of wheat either by mouth or by subcutaneous injection without asthma. While on a wheat-free diet without other treatment the patient was practically free from symptoms including indigestion, gas, and the feeling of fulness after a hearty meal. Following this prolonged wheat-free diet the patient's skin reactions were markedly reduced since the wheat and other cereal proteins now gave only slightly positive reactions. The change in the character and frequency of the patient's asthma after an operation for appendicitis and the abnormal conditions in the patient's gastro-intestinal tract as revealed by bismuth studies are of interest.

Case 21. — J. D., a baker aged 40, was first seen on Sept. 23, 1916.

The patient has had asthma for only one year although he has been a baker for sixteen years. The first attack began with a cough and he has had a cough ever since. He has asthma only when he is working in the bakeshop.

Cutaneous reactions were positive with the wheat proteins in the following dilutions: natural proteose, globulin, and leucosin in dilutions of 1–1,000, glutenin and gliadin 1–100, whole wheat 1–500; the artificial proteose gave a doubtful reaction in concentrated amounts.

Treatment was given subcutaneously at weekly intervals with three and five minims of a mixture of the wheat proteins in dilutions of 1–10,000; during this time he was practically free from symptoms. Following eight minims he had a relapse and he was as bad as ever. The patient was then put on a wheat-free diet and was given subcutaneously three minims of the wheat proteins in a dilution of 1–10,000. During this week the patient was fine. The next week he was allowed to eat half as much wheat as usual, was given three minims of the wheat proteins in a dilution of 1–10,000 as in the previous week. Following this treatment he had asthma as usual. Since then the patient has been on a nearly wheat-free diet without other treatment and he has had practically no symptoms although he continues to bake as usual.

Summary. — This patient illustrates the difficulty in desensitizing with the wheat proteins. He was unable to both eat and inhale wheat without asthma, cough, and wheezing, and the same was true of either eating and being injected with wheat and of inhaling and being injected with wheat. He could, however, inhale wheat while on a wheat-free diet and when he was not injected with it. In other words, he was

free from symptoms when small amounts of wheat protein entered his body and it made no difference how it entered, but when larger amounts entered his body no matter in what way he had asthma.

Case 22. — G. B., a baker aged 52, was first seen on Oct. 4, 1916.

The patient has had asthma for nine years. He has it much worse in winter, when wheezing and cough interrupts his sleep, and he cannot walk without getting out of breath and having to rest. He takes cold easily and he has asthma with colds. He is no worse when working with flour than at other times. He has had no spasmodic asthma, but he wheezes all of the time and has a tightness across the chest. He has emphysema of the lungs, a little albumen in his urine, and a blood pressure of 147–70.

Cutaneous reactions were positive with the whole protein of corn, rice, barley, oat, and wheat; they were slightly positive with the proteins of wheat and they were positive with corn and the wheat proteins, natural proteose and globulin, in dilutions of 1–100.

Treatment: The patient was given five subcutaneous injections with the protein of corn and the wheat proteins, globulin and natural proteose, each in a dilution of 1–1,000 without improvement. He was then put on a wheat-free diet with no improvement, and he was no worse when he again began to eat wheat. During the next three months the patient was given a vaccine made from the predominating organism (a diphtheroid) in his sputum. After a few vaccine treatments the patient was able to sleep without being waked by wheezing and cough, he became able to walk without trouble and he felt better. In contrast to the way he felt the previous winter he was now very much better and he was having little trouble.

Summary. — Although the patient was sensitive to the proteins of the cereals, they seemed to play no part in the cause of the patient's symptoms at this time. The history and examination of the case would seem to indicate that he had little asthma, but chiefly chronic bronchitis and emphysema together with a little disturbance of the kidneys. Vaccine treatment with the predominating organism in his sputum certainly gave him a much more comfortable winter than he had had for years.

Case 23. — F. G., a housewife aged 42, was first seen on Oct. 19, 1916.

Family history: The patient's father had asthma, and a sister has attacks of watering of the eyes, running of the nose, sneezing and coughing when baking, but at no other time.

Present illness: The patient has had asthma for over twenty years. The first attack came in the winter and began with shortness of breath, coughing, wheezing and expectoration, much like a severe cold. During the first few years attacks came every two or three months and each attack was of three or four days' duration. During the past few years the patient has had no real severe asthma, because she constantly uses an atomizer which she says prevents real attacks, but she has tightness across the chest on exertion, on exposure to dust, cold air, wind, and changes in the weather. She has little cough and no expectoration. She has more wheezing with colds than at other times.

Cutaneous reactions were positive with wheat leucosin in a dilution of 1–100 and they were slightly positive with wheat globulin, gliadin and natural proteose in a concentrated form. On repetition of the skin tests three months later wheat leucosin in a dilution of 1–100 still gave a positive reaction.

Treatment: Six subcutaneous injections of wheat leucosin in a dilution of 1–1,000 were given in gradually increasing amounts and during the last three weeks of this treatment the patient was on a wheat-free diet. No improvement followed. The next two weeks the patient was on a wheat-free diet with no other treatment without improvement and on resuming the wheat diet the patient was no better or no worse. Throughout the next two months the patient was treated five times with a stock vaccine made from a diphtheroid organism and six times with a vaccine made from the predominating organism (a diphtheroid) in her own sputum. During these vaccine treatments the patient showed no marked improvement, although from week to week there was slight improvement in that she was able to walk better and farther without wheezing and she did not use her atomizer nearly as frequently as before. As further evidence of improvement the patient had always had much wheezing or asthma with a cold, but during vaccine treatment she had a cold for two weeks without any wheezing. Following two treatments with S. pyogenes aureus and albus stock vaccine in addition to the autogenous diphtheroid vaccine she had bad asthma, a sore arm, felt stuffed up, and used her atomizer more frequently. Since this was the only occasion in the course of the treatment when the patient was distinctly worse, it would seem that treatment with vaccines which contain several types of organisms was detrimental to the patient, but that treatment with vaccines made from one type of organism, which in this case was the predominating one in the sputum, resulted in some improvement.

Summary. — Although this patient gave a positive skin test with wheat leucosin, since the positiveness of this test did not decrease and since the patient did not improve while under treatment with leucosin or while on a wheat-free diet, there is no other evidence that wheat leucosin at the

present time is the cause of asthma in this patient. The result of treatment with vaccines of the predominating organism in the patient's sputum is quite indefinite: although the patient did not use her atomizer as frequently she could walk better and farther than before without wheezing, and she did have a cold without asthma. There is no question, however, but that treatment with vaccines made from several types of organisms was followed by making the patient worse.

Group IV.

Patients Sensitive to Miscellaneous Proteins.

Case 24. — H. T., a girl aged 16, was admitted to the medical ward on May 1, 1916, medical number 4586. A diagnosis of bronchial asthma was made.

Family history: The patient's grandfather had asthma and her father and a brother have asthma.

Present illness: The patient has had asthma since six months of age; attacks usually begin each September and continue off and on until summer. Attacks are usually of two to three days' duration, but occasionally one continues for two weeks and rarely for two months. She often wakes up at night with asthma. Attacks used to be accompanied with much cough and expectoration, but during the past seven years she has had nausea and vomiting with asthma. She associates asthma with attacks of bronchitis and tonsilitis and with being overtired. She has very large tonsils and two very bad teeth.

Cutaneous reactions were positive with alcoholic extracts of horse dandruff, cat, dog, and cattle hair; the serum protein of these animals gave negative tests. The protein of chicken meat gave a positive test.

Treatment: The family cat was disposed of, the patient's teeth were put in good condition, and she was advised to avoid petting dogs and eating chicken. The patient was free from asthma for six months until January, 1917. During January and February the patient had two slight attacks of asthma; one came about an hour after she had played with a cat and the other came about two hours after she had played with a dog; there was a six weeks' interval between these attacks. Recently the patient has had more or less asthma during an attack of tonsilitis, but she refuses to have her tonsils removed.

Summary. — During the first half of the winter the patient was free from asthma, and she avoided cats, dogs, and eating chicken. During the next two months she had two attacks of asthma, one followed exposure to a cat and the other six

weeks later followed exposure to a dog. Recently she has had asthma with tonsilitis, but she refuses to have her tonsils which are large removed. This case seems to have been improved by following the results of the skin tests, since she has had a winter of practical freedom from asthma, whereas during previous winters she has had frequent attacks of asthma which incapacitated her for most of the winter. She has been free from nausea, vomiting, and cough.

Case 25. — A. C., a schoolboy aged 15, was admitted to the medical wards on Oct. 14, 1915, medical number 3459. A diagnosis of bronchial asthma was made.

The patient has had asthma for four years. He associates attacks with exertion, excessive eating, and windy weather; he has more asthma in winter than in summer. The attacks usually come at night and continue for only a few hours; he becomes free from the attack in the morning.

Cutaneous reactions were repeatedly positive with the protein of salmon and chicken meat.

Treatment: The patient was required to keep a diary of everything he ate and did so for a period of two months. During this time each attack of asthma had followed eating either salmon or chicken. During the next six months the patient was allowed to eat salmon or chicken on six different occasions and each time he had more or less asthma and had no asthma at any other time. For three months the patient ate neither salmon nor chicken and during this time he had asthma only once when out in a strong wind. At present the patient is a member of the track and swimming teams at school and has no trouble on exertion. Recently the patient has had asthma on Sundays only, and on this day he is accustomed to stay in the house and smoke all day. The next three Sundays the patient was required to take a long walk or to work, and on these days he had no asthma.

Summary. — Chicken meat and salmon would seem to have been the cause of most of the attacks of asthma in this case. The recent Sunday night attacks may have been due to lack of exercise, since on week days the patient was very active in athletics or it may have been due to tobacco smoke in a closed room.

Case 26. — W. M. O'D., a car conductor aged 26, was first seen on Oct. 27, 1916.

Family history: The patient's maternal grandfather had asthma.

Present illness: The patient has had asthma for three years, the first attack began with a severe cold. Each attack usually continues for three

or four days and he is free for four or five days. He takes cold easily and has a cough and much expectoration all of the time. He associates asthma with colds, changes in the weather, and dampness. He has asthma more than he is free from it and he has to burn an asthma powder three or four times every night, and in spite of this he has an attack of wheezing and coughing every morning. He has an inguinal hernia.

Cutaneous reactions were positive with the protein of casein, potato, and S. pyogenes aureus.

Treatment: During three weeks of a milk-free diet the patient had no attacks of asthma, but he continued to wheeze and cough as usual. He then was put on a milk and potato-free diet for two weeks and he had less wheezing and cough. Continuing this diet he was in addition given at weekly intervals subcutaneous injections of a 1–100 casein solution for four weeks, and he continued to have wheezing and cough every morning, although he burned his powder only at this time. During the next six weeks the patient was given a stock S. pyogenes aureus vaccine in conjunction with the casein solution; he became more free from wheezing and cough, but he had one attack of asthma for three days. After this the casein solution was discontinued, the S. pyogenes aureus vaccine was continued, and a stock diphtheroid vaccine was given in addition; and two weeks later on a stock S. pyogenes albus vaccine was added. This combined vaccine treatment was given six times at weekly intervals. During this time the patient had two attacks of asthma, each of one day's duration, but between these attacks he was entirely free from wheezing and cough and no longer burned his powder.

Summary. — Casein and potato would seem to have played some part in the cause of asthma in this case since he had no attacks for several weeks while he did not eat these foods; however, he continued to wheeze and cough most of the time. Subcutaneous injections of a casein solution gave no relief. Vaccine treatments with S. pyogenes aureus in conjunction with casein solution did seem to give some relief. Vaccine treatment with S. pyogenes aureus, diphtheroid, and S. pyogenes albus all combined gave the most relief, since he had only two attacks of asthma each of one-day duration in six weeks, and he became free from wheezing, cough, and expectoration the remainder of the time. One cannot justly say which vaccine helped the most. During all this treatment the frequency of the attacks of asthma had been diminished, since previous to treatment he averaged an attack every few days, but during treatment he averaged an attack every three weeks. The severity of attacks was also greatly

diminished, since he had usually been laid up for a half of each week, but during treatment he was away from work for only a day or two at a time. The patient's recent short attacks of asthma have been associated with vomiting and indigestion and it is a question how much the patient's hernia has to do with these attacks, since he wears no truss, and he complains about the dragging and tired feeling produced by the hernia after being on his feet all day. He no longer associates asthma with colds and weather changes, but more with vomiting attacks.

Case 27. — A. G. C., a nurse aged 28, was first seen on March 8, 1916.

Family history: The patient's oldest sister and an uncle on her father's side have asthma.

Present illness: As a child the patient had bronchitis every winter for several years and for bronchitis flaxseed poultices were used. Seven years ago while making a flaxseed poultice the patient had an attack of sneezing, coughing, watering of the eyes, and severe dyspnea; a physician called this an attack of asthma and the patient now thinks that she probably had asthma as a child with the bronchitis. This winter on four different occasions the odor of flaxseed poultices made her choke up, but she succeeded in getting away from them without further trouble. Recently at this hospital on going into the room where a flaxseed poultice had just been made the patient had an attack of asthma of twenty minutes' duration.

Cutaneous reactions were positive with various extracts of ground flaxseed; from an aqueous extract of ground flaxseed a protein was prepared by precipitation with acetone and washing with alcohol and ether. A 1–10,000 dilution of this protein gave a positive skin reaction in seven minutes.

Treatment: The patient was given subcutaneously .000125 gram of the protein, which was precipitated by acetone and washed with alcohol and ether. Five minutes later the patient began to cough and choke. Five minutes later still the patient could hardly walk because of dyspnea, her head became very hot, and her collar felt too tight. Two minutes later her neck began to itch terribly, her eyelids began to swell, and very shortly the right eye was closed. Her head began to ache severely, her ears felt like bursting, her heart felt queer, she was sick at her stomach, she had great difficulty in breathing, and she felt as if she was going to die. At this time the patient's fingernails and lips were blue and her conjunctivæ were greatly injected. Large white urticarial wheals began to appear over the body down to the waist line, one wheal reached from the elbow to the wrist, all had large red areolæ around them and they itched terribly. The distal phalanx of each finger was snowy white and the

remainder of the fingers and the whole hand was red and hot. As the wheals gradually disappeared from the upper part of the body more appeared on the lower part and these in turn gradually disappeared until about an hour later the whole body was free from them. During the height of the dyspnea and while the wheals were appearing on the upper part of the body, one cubic centimeter of adrenalin chloride in a dilution of 1–1,000 was given subcutaneously; this promptly relieved the headache, the earache, and the dyspnea. Although the patient felt dead tired, as though she had done a very hard day's work, she was unable to sleep for two hours, and then only for a few minutes, because of severe gas pains in her abdomen. Later the patient was able to sleep and the next morning she got up early feeling normal and went to work as usual.

Summary. — This case is interesting in that it shows sensitization to an unusual protein, that a very small amount of this protein caused a severe reaction, and that the train of symptoms was followed closely. Three different manifestations of protein sensitization were present in this case following one injection of protein: namely, asthma, urticaria, and intestinal disturbances in the form of severe gas pains.

Case 28.— A. A. D., a woman aged 49, was first seen on Oct. 2, 1916.

Family history: The patient's sister and paternal grandfather had asthma.

Present illness: The patient had asthma in winter between the ages of eight and eleven. The next attack of asthma came in April, 1916, with a cold and it continued for a week. Since this attack the patient has had a little asthma off and on and during the past month she has been choked up and short of breath every night after being in her room for awhile, and each morning at about three or four o'clock she has had real asthma. The patient has noticed that tobacco smoke caused her to choke up and that the dust from feather pillows causes her to wheeze. She uses extra coverings over her pillows to avoid pillow dust.

Cutaneous reactions were positive with an alcoholic extract of feathers and with an alkaline extract of tobacco.

Treatment: On careful questioning it was learned that a man who smokes continuously occupies the room adjoining to and communicating with her own, and that the patient has been living in this house only for the past month, during which time she has had asthma every night. Since the patient noticed the odor of tobacco smoke in her own room and since she choked up in the presence of tobacco smoke, the patient was advised to change her room to a place where there were no smokers. She did this and has had no wheezing or asthma since then, which was four months ago.

Summary. — Tobacco smoke would seem to have been the cause of asthma in this case.

Case 29. — P. J. F., a clerk aged 48, was first seen on Aug. 18, 1916. The patient has had asthma for twelve years and preceding the asthma he had hay fever for three years. The first attack of asthma began following bronchitis and he has had asthma and bronchitis ever since; he has asthma more than he is free from it. He has colds on the chest and asthma with every change of the weather, and he has a cough with profuse expectoration most of the time. Raising sputum seems to bring relief from asthma.

Cutaneous reactions on August 18th could not be depended upon because the patient's skin was so sensitive and irritable that every protein which was tried gave a positive reaction. On August 23d they were repeated with similar results. On November 22d cutaneous reactions were again repeated with similar results.

Treatment: From August 23d to November 8th the patient was given at weekly intervals stock vaccines of S. pyogenes aureus in gradual increasing amounts from 200 to 650 million. This method of treatment was followed because the patient's serum agglutinated this organism. Following the first two treatments the patient had a bad attack of asthma for three days, such as he has been accustomed to have. During the remainder of the treatment which continued for two months the patient had asthma only on one day although he continued to wheeze with colds, exertion, and changes of weather, and his cough and expectoration were somewhat diminished. Further treatment was discontinued and instead for the next three months the patient received osteopathic treatment with little or no improvement in the asthma.

Summary. — This patient illustrates the occasional deception of the cutaneous reaction in patients who have an irritable skin and the necessity of carefully controlling the tests by using a large number of them at one time together with normal controls. The positive agglutination between the patient's serum and S. pyogenes aureus was the only positive lead to treatment in this case and vaccines with this organism gave some relief.

Group V.

Patients Sensitive to the Protein of S. Pyogenes Aureus and Treated with Vaccines of this Organism.

Case 30. — M. L S., a dressmaker aged 31, was admitted to the medical ward on April 6, 1916, medical number 4467. A diagnosis of bronchial asthma was made.

Family history: The patient's brother and a maternal first cousin have asthma, and her maternal grandmother had asthma.

Present illness: As a child the patient had frequent colds and attacks of bronchitis. Between the ages of twelve and fifteen she had attacks of asthma with colds, but between the ages of fifteen and seventeen she was free from both asthma and colds. In the fall of her seventeenth year the patient had a bad cold and asthma, both of which she has continued to have ever since. At first the patient had four attacks of asthma each year, one in the fall, one in the spring, and two in the winter, and each attack would continue for four days. Later, she began to have indigestion and gas on her stomach with attacks of asthma; onions, turnips, beans, cabbage, lettuce, oranges, and uncooked fruit all seemed to cause indigestion, gas, and asthma. More recently she has had eczema with severe attacks of asthma. During the last four months she has had headache and vomiting with attacks of asthma which now come more often at night. Cold air, dust, wind, and exertion provoke wheezing, and she has had asthma in half an hour after strong irritating odors. Asthma and menstruation seem to come together and she had asthma all the time that she was pregnant and at childbirth. She has to burn an asthma powder every day or night and frequently several times.

Cutaneous reactions were positive with the protein of S. pyogenes aureus. Her serum agglutinated this organism.

The patient was discharged from the hospital on April 19th without treatment and was advised to have two decayed teeth treated. She had an attack of asthma for three days in April and another for two weeks in May.

Treatment was begun on May 17th with 200 million of stock S. pyogenes aureus vaccine, on May 23d 350 million were given and a week later 500 million were given. Following each of the last two treatments the patient had wheezing for two days, so that the treatment the following week was reduced to 200 million. During the next two months the patient was given at weekly intervals gradually increasing amounts of this vaccine, beginning with 200 million and ending with 1,000 million. During this time she had no wheezing or asthma, she was free from indigestion, was able to work every day, and dust, wind, and exertion caused no trouble. Her serum no longer agglutinated S. pyogenes aureus. The patient was not seen for three months, during which time she had continued free from all symptoms, although she had had a cold and bronchitis for two weeks. On November 10th she reported that she had had a cold for the past few weeks, and for the past week she had had asthma, cough, and much expectoration; her serum now agglutinated S. pyogenes aureus. Treatment was begun again with stock S. pyogenes aureus vaccine and continued as previously from November 10th to March 12th. During the first month of treatment she had slight wheezing at times, and the cough and expectoration gradually decreased, and her serum soon failed to agglutinate S. pyogenes aureus. During the last three months of treatment she was free from all symptoms.

Summary. — During treatment with stock vaccines of S. pyogenes aureus this patient became free from asthma, cough, and expectoration, and remained free for five months, during three months of this time she was given no treatment. When asthma returned again with a long-continued cold, she was again relieved of it by similar treatment, and remained free for three months. It is noted that permanent results did not follow treatment, but a relapse followed after three months without treatment. It is interesting that this patient associated asthma with indigestion and gas caused by certain foods, but that successful treatment relieved her of these symptoms, even though she ate these same foods. During the first series of treatments the patient did not seem to do well when the vaccine was rapidly increased, but when more slowly increased she quickly improved. The agglutination tests with this patient's serum were interesting.

Case 31. — E. O'B., a woman aged 24, was first seen on Aug. 4, 1916.

Family history: The patient's grandfather had asthma.

Present illness: Thirteen years ago the patient had whooping-cough and following this she got short of breath easily and soon she had real asthma. Attacks occur at all times of the year and at first she had frequent colds with asthma, but since the removal of a spur and a polyp from the nose she has had fewer colds, but just as much asthma. Each attack of asthma used to continue for two or three weeks, but during the past two years they have been of two or three days' duration. Two years ago she averaged an attack every two weeks, but the past year she has had asthma about every week. She associates asthma with fatigue, exertion, indigestion, and overeating. A year ago she had rheumatism of the large joints.

Cutaneous reactions were negative on the day the tests were made, but the following day the cuts which were inoculated with the protein of S. pyogenes aureus, S. hemolyticus, and a diphtheroid organism were red, sore, and a little inflamed. Her serum agglutinated S. pyogenes aureus.

Treatment was given at weekly intervals for seven weeks with a vaccine made from a stock diphtheroid organism in doses varying between 400 and 750 million bacteria. During this time the patient continued to have asthma every week as previously, some weeks for only two days, and other weeks for six days; she was not improved. On October 2d treatment was changed to a stock vaccine of S. pyogenes aureus; this was given at

weekly intervals with gradually increasing doses from this time to March 6th. During these five months the patient was greatly improved; she had asthma on only two days and four nights, occasionally she would wheeze for a few hours and her cough stopped. She has worked every day this winter, and wind, dust, exertion, and overeating no longer trouble her, in fact she has not been prevented from doing anything she wished to do.

Summary. — This patient would seem to show a specificity in bacteria in the treatment of asthma since she did not improve when treated with a diphtheroid vaccine, but did have practical relief when treated with S. pyogenes aureus vaccine. Last year she was laid up half the time, this year she has not been laid up or prevented from work but a few days. The patient illustrates a delayed positive cutaneous reaction with bacterial protein.

Case 32. — R. G. V., a schoolboy aged 16, was admitted to the medical wards on Sept. 29, 1916, medical number 5373. A diagnosis of bronchial asthma, chronic bronchitis, and emphysema was made.

Family history: A paternal aunt has asthma.

Present illness: The patient has had asthma for ten years; the first attack was with a cold and bronchitis. During the first few years he had infrequent attacks of asthma, three years ago he averaged an attack every two weeks, and last year he had five attacks of long duration. He has a severe attack of asthma every fall. He has frequent head colds and he coughs and raises sputum most of the time. He associates asthma with colds, windy weather, exertion, indigestion, and following the eating of lobster.

Cutaneous reactions were repeatedly positive with the protein of S. pyogenes aureus and a diphtheroid organism and on the day after each test these places were red and inflamed. The protein of lobster and the pollen of rag weed and of daisy gave positive reactions.

Treatment was given at weekly intervals with a stock vaccine consisting of equal parts of S. pyogenes aureus and diphtheroid organisms from October 4th to January 6th; the dose was gradually increased from 200 million to 1,000 million. During this time the patient had asthma only on one day, which was early in the treatment; exertion, winds, and changes in weather no longer trouble him and he has been free from colds, indigestion, and asthma. The skin reaction with these two bacterial proteins became almost negative. It has been two months since treatment was discontinued and he is still free from all symptoms.

Summary. — This patient seems to have been greatly benefited by vaccine treatment with the organisms to which

he gave a positive skin reaction with their proteins. It is interesting that with a history of indigestion and asthma following the ingestion of lobster he should give a positive cutaneous reaction with the protein of lobster.

Case 33. — M. M., a housewife aged 44, was first seen on Sept. 13, 1916.

Family history: The patient's mother and maternal grandfather had asthma.

Present illness: The patient had hay fever between the ages of fifteen and thirty and she has had asthma ever since the age of thirty. For the first few years she had only two attacks of asthma during the year, these sometimes came in winter and sometimes in summer. Later on she had frequent colds on the chest and she had asthma with every cold. During the past three years the patient has had asthma every night and often during the day, and she would sometimes be confined to her bed for weeks at a time. She has to burn an asthma powder several times every night, and some nights she burns it every few minutes; she coughs most of the time and raises thick sputum which seems to give some relief. During the past six weeks she has been confined to her bed and she was brought to the hospital for the skin tests in a very weak condition, and then she was confined to her bed for three weeks more.

Cutaneous reactions were positive with the protein of S. pyogenes aureus, slightly positive with that of S. pyogenes albus, and positive with the pollen of red top, timothy, and rose. The patient's serum agglutinated strains of S. pyogenes aureus.

Treatment had to be carried out by the family physician since the patient was sick in bed. The patient was given respectively 100, 200, 200, 250, and 300 million of S. pyogenes aureus stock vaccine at weekly intervals. After the third treatment the patient began to improve and she was able to come to the hospital for the sixth treatment on October 13th. From this time until February the patient was treated with gradually increasing amounts of stock S. pyogenes aureus vaccine, the last dose consisted of 750 million bacteria. During this time the patient had slight attacks of wheezing of a few minutes' duration once or twice each week and at these times she burned her asthma powder, but this was not a regular occasion. She had one cold and with it some asthma for two days. She was able to entertain, to be out late at night, and to be exposed to cold night air without asthma, all of which she has been unable to do for years. Since the patient still had slight attacks of wheezing at times for which she burned her asthma powder, it seemed best to treat the patient with stock S. pyogenes albus vaccine. She was given 200 million of this and she had an attack of asthma for three days. The following week the same dose was repeated and the patient had asthma which confined her to her bed for two weeks and she had to burn her asthma powder every

few minutes; in other words, she was as bad as before treatment was begun with S. pyogenes aureus vaccine. Treatment was again resumed with the latter and the patient is gradually improving again as before.

Summary. — There would seem to be no question but that stock vaccines with S. pyogenes aureus greatly improved this patient, as she was practically free from asthma for four winter months and treatment was begun when she was confined in bed with asthma. Two treatments with stock vaccines made from S. pyogenes albus seemed to upset all improvement, since following these two treatments the patient became sick in bed with asthma. We have observed several similar experiences with other patients, so that there would seem to be a specificity in the type of bacteria which should be used in the treatment of asthma and any type of bacteria will not suffice. This patient again began to improve when treatment with S. pyogenes aureus vaccine was again resumed.

Case 34. — G., a physician's wife aged 30, was admitted to the medical ward on May 16, 1916, medical number 4669. A diagnosis of bronchial asthma was made.

Six months ago the patient had tonsilitis and a cold on her chest and since then she has continued to cough and to have a tightness across her chest with whistling in her chest on exertion, in wind and in cold air. Three months ago she noticed dyspnea on slight exertion and during the past month or two she has had attacks of asthma of half an hour duration, chiefly at night; she often wakes up with asthma and a tightness across her chest. She has a bad cough with profuse expectoration.

Cutaneous reactions were positive with the protein of S. pyogenes aureus.

Treatment has been carried out by the patient's husband, who is a physician. During the next four months the patient was given at irregular intervals of usually ten days gradually increasing amounts of a stock vaccine made from S. pyogenes aureus. The patient gradually improved until she was free from asthma under all conditions, but she still had a little morning cough; she said that she felt like a new person. Treatment was discontinued. Three months later the patient had a return of heaviness in her chest, difficulty in breathing, tired feeling, and although she had no real asthma she wheezed all of the time. Treatment was begun again with the same vaccine and after three doses she became free from all symptoms with the exception of an occasional attack of wheezing which cleared up after three more treatments.

Summary. — This patient seems to have been relieved by S. pyogenes aureus vaccine, although it took a longer time than with most cases, possibly because the vaccine was given at irregular and infrequent intervals. Three months after treatment was discontinued the patient began to relapse, but on resumption of treatment she again became free from symptoms.

Case 35. — A. P J., a farmer aged 38, was admitted to the medical wards on March 15, 1916, medical number 4328. A diagnosis of bronchial asthma was made.

Family history: Two maternal aunts of the patient had asthma.

Present illness: Asthma began three years ago with cough and profuse expectoration. Attacks of asthma seemed to be relieved by raising sputum and they are caused by exertion, colds, cold air, and a full stomach; it matters not whether the patient's stomach is filled with gas, food, or water, asthma follows just the same. Three years ago following the removal of nasal polyps asthma was relieved for a short time.

Cutaneous reactions were positive with the protein of S. pyogenes aureus and of spinach.

Treatment was carried out by the patient's family physician. For the next two months the patient was given at ten-day intervals gradually increasing amounts of a stock S. pyogenes aureus vaccine. During this time the patient stated that he had felt better and had been more free from asthma than for three years and that strenuous exertion was the only thing that now caused him to have asthma. The patient was not heard from again until November, when he said that the asthma was again troubling him at night. He had continued free from colds. On resuming the vaccine the patient again became free from asthma, except following strenuous exertion and on exposure to very cold air.

Summary. — The physician who gave this patient the vaccine writes as follows: " I am of the opinion that the vaccine helped the patient very much."

Case 36. — R. B., a housewife aged 69, was first seen on Oct. 30, 1916. The patient has had asthma for thirty years or more. Attacks come chiefly in the spring and fall and they are associated with colds. She wheezes all the year round and damp, foggy weather, windy weather, and cold air make her wheeze more; walking, going up stairs, and exertion cause marked wheezing and dyspnea. She has a constant cough with much expectoration, both of which are worse at night and interfere with sleep.

Cutaneous reactions were positive with the protein of S. pyogenes aureus.

Treatment was carried out by her family physician at home. The patient was given gradually increasing amounts of stock S. pyogenes aureus vaccine at weekly intervals. The amount varied between 200 and 500 million bacteria. The patient was not seen for two months. At this time she reported that she no longer coughed at night, she was able to walk up three flights of stairs without wheezing, windy and damp weather no longer affected her breathing, and she was free from wheezing most of the time. She had had no real asthma.

Summary. — This patient seems to have been markedly improved by S. pyogenes aureus vaccines.

Case 37. — W. C. T., a pedler aged 45, was admitted to the medical wards on Aug. 21, 1916, medical number 5161 ; on Sept 18, 1916, medical number, 5316; on Dec. 1, 1916, medical number 5694; and on March 10, 1917, medical number 6267. On each admission the same diagnosis was made, chronic myocarditis, chronic nephritis, hypertension, chronic bronchitis, emphysema, and in addition on the first two visits a diagnosis of bronchial asthma was made.

Two years ago the patient began to have spells of coughing on lying down at night and on getting up in the morning and shortness of breath on going up stairs. Later he began to wheeze and shortly after this he began to have real asthmatic attacks at night. He had to burn an asthmatic powder every night for relief. During the past year he has had to sit up every night to get his breath, and frequently he wakes up with asthma. The patient has just spent two months in another hospital.

Cutaneous reactions were negative on the day they were made, but the next day the cuts which had been inoculated with the protein of S. pyogenes aureus were swollen, red, and inflamed; these were called positive tests. The patient's serum agglutinated strains of S. pyogenes aureus.

Treatment: The patient was given two treatments with a stock vaccine made with a diphtheroid organism while he was in this hospital, and two after he was discharged. Since he was not improved and had developed general anarsaca he was readmitted to the hospital on September 18th, complaining of the same symptoms as previously described. He was given 200 million of stock S. pyogenes aureus vaccine and from the next day on he had no asthma. This treatment was continued at weekly intervals with gradually increasing amounts of the vaccine for four months. During the first two months he was free from asthma and his cough and expectoration rapidly decreased until he was able to work and go up stairs without wheezing During the third month he was readmitted to the hospital because of edema and dyspnea, but he claimed that he still had no asthma and during his three weeks' stay in the hospital he was free from asthma and wheezing. After being discharged from the hospital he continued free from asthma and practically free from cough for another two months, although vaccine treatment had been discontinued for one month.

Summary. — Previous to the present studies this patient had been under observation for a period of two months in another hospital where it was thought that the patient's heart condition was possibly secondary to his asthma; hospital treatment had improved his general condition, but not his asthma. Neither was the patient's asthma improved by his stay in this hospital or by treatments with a stock diphtheroid vaccine. Following one treatment with a stock vaccine of S. pyogenes aureus the patient became free from asthma and his cough and expectoration greatly decreased, although there was no improvement in his edema or heart symptoms. Treatment with S. pyogenes aureus vaccines was continued for four months and during this time the patient was free from asthma and had very little cough, although he had a return of edema and cardiac symptoms which necessitated hospital treatment. A month after vaccine treatments were discontinued the patient began to have a little asthma at night with a bad cough, and edema and cardiac symptoms again returned so that he was readmitted to the hospital. In this case the cause of asthma would seem to be independent of his heart and kidney condition and asthma would seem to have been due to S. pyogenes aureus, since the patient was relieved of asthma and remained free from it for over four months during treatment with stock vaccines of this organism. This patient illustrates the delayed type of positive cutaneous reaction with bacterial protein. Such a reaction is not uncommon with these proteins.

Group VI.

Patients Sensitive to the Protein of S. Pyogenes Albus and Treated with Vaccines of this Organism.

Case 38. — A. A. B., a physician aged 40, was first seen on June 21, 1916.

Family history: The patient's maternal grandfather had asthma.

Present illness: The patient has had asthma for one year and previous to this he had had bronchitis for two months. He has asthma "spasms" several times a day and these are worse at night so that his sleep is broken by them. He has a constant bad cough and he frequently raises a half pint of sputum daily. He takes several asthma "cures" and potassium iodide constantly, which seems to check the asthma "spasms" somewhat.

Cutaneous reactions were positive with the protein of S. pyogenes albus. These tests were made by Dr. J. L. Goodale of Boston.

Treatment: Since the patient is a physician and lives out of town, he has treated himself with our vaccines, and he has communicated to us the results of treatment. During four weekly injections with large amounts of S. pyogenes albus vaccine, the patient was free from asthma "spasm," and his cough and sputum decreased a good deal, he felt better and stronger. During the next two months he experimented with himself and came to the conclusion that small amounts of the vaccine (200 to 300 million) taken every four days kept the amount of his cough and sputum at a minimum better than large amounts taken at weekly intervals, although either mode of treatment kept him free from asthma "spasm." He was then given a stock diphtheroid vaccine, but his cough, sputum, and asthma spasm returned. On resumption of the stock S. pyogenes albus vaccine the cough and sputum again decreased and the asthma left him. He was then given a vaccine made from his own sputum for a few treatments. This seemed to decrease the amount of cough and sputum more than did the albus vaccine, but it did not control his asthma spasm. On returning to the stock albus vaccine he became free from asthma and has remained so, and his cough and sputum no longer interfere with sleep and work.

Summary. — The patient's own words are as follows: "I think that the S. pyogenes albus vaccine has done wonders for me. I feel a great deal better and stronger. I have no asthma and the cough and sputum are at a minimum. I find that the albus vaccine taken every four days in amounts of 200 or 300 million has done better than the same vaccine taken at weekly intervals in larger amounts. I could see no improvement from the diphtheroid vaccine; in fact, I was less free from symptoms while taking it. The sputum vaccine seemed to clear up the amount of cough and expectoration, but it did not control the asthma. Albus vaccine is the only thing that has relieved me, but I find that I cannot stop it for more than a month without going backward."

Case 39. — F. S. E., a business man aged 35, was first seen on May 18, 1916.

The patient has had asthma for two years. Attacks come chiefly in the winter, and are preceded by colds. Each attack usually continues for about an hour, then he coughs and raises thick sputum which seems to bring relief. He has a bad cough most of the time, and his sleep is broken by coughing and wheezing. Exertion, dust, and getting overtired cause wheezing.

Cutaneous reactions were positive with the protein of S. pyogenes aureus and albus. The patient's serum agglutinated S. pyogenes aureus.

Treatment was given at weekly intervals with stock vaccines of S pyogenes albus, 200, 400, 500, and 600 million bacteria were given respectively four times. The patient now felt better, his sleep was unbroken, he no longer had asthma or wheezing, he coughed only in the morning, and his breathing felt easier. The patient was not seen again for seven months until Jan. 16, 1917. He reported that he had been free from all symptoms except his morning cough and some expectoration up to four weeks ago, when he had a severe cold with much cough and expectoration, and two weeks ago he began to have asthma every morning. He was given five treatments at weekly intervals with S. pyogenes albus stock vaccine, 250, 300, 350, 400, and 400 million bacteria respectively. Again he became able to sleep without difficulty, the cough and expectoration greatly diminished, and he had only two attacks of asthma of ten minutes' duration each. Again the patient stopped treatment because he thought he was so much better.

Summary. — Treatment with stock vaccines of S. pyogenes albus seemed to help this patient a great deal on two different occasions. He felt so much improved, and was so nearly free from symptoms that he voluntarily discontinued further treatment.

Case 40. — I. G., a housewife aged 37, was first seen on Nov. 15, 1916.

The patient has had asthma for ten years. Attacks usually continue for one day, but occasionally for ten days, and she has them at irregular intervals, but more frequently in the winter. She takes cold easily, and she associates asthma with colds, overeating, exertion, and dust.

Cutaneous reactions were repeatedly positive with the protein of S pyogenes albus, and with the pollen of rag weed, goldenrod, red top, timothy, and daisy.

Treatment was given from November 15th to March 1st with a stock vaccine of S. pyogenes albus; each week the dose of the vaccine was gradually increased from 200 million the first week to 1,000 million the last week. During the first five weeks of treatment the patient had a little wheezing on two nights, and she frequently choked up when suddenly exposed to cold air. During the remainder of the treatment the patient had no trouble from dust, cold air, or exertion, and she had no cold. This has been the first winter that she has been free from colds. On two occasions, following a late supper which consisted chiefly of honey with a little bread, she had wheezing.

Summary. — This patient seems to have been benefited by stock vaccine of S. pyogenes albus, since she has been

free from her usual winter colds, and exposure to dust and cold air no longer cause her to wheeze, and she has been free from asthma with the exception of wheezing after eating honey. Cutaneous reactions were negative with a drop of this honey as one would expect, since it would contain only traces of protein, but skin tests were positive with the pollen of several plants, and this pollen we know contaminates through mechanical means other pollens from which bees make honey, and thus the honey itself might be contaminated by the pollen to which she was sensitive. Another patient whom we have studied has asthma after eating honey.

SUMMARY.

Group I. consists of nine patients who were sensitive to and who were treated with the proteins found in horse dandruff. Case 1, D. G., is of interest in that previous to an injection of antitoxin the patient had had no asthma and was not sensitive to horses, but two weeks after the antitoxin she began to have asthma and exposure to horses gave her asthma; she was sensitive to the proteins found in horse dandruff, but not to those in horse serum. The cutaneous reactions in this case would seem to show a difference between the separate proteins in horse dandruff. The patient illustrates a variety of symptoms since the onset of asthma, all of which were relieved following subcutaneous injections with horse dandruff alkali meta-protein, and the positiveness of the skin reaction was diminished one hundred-fold. The case also illustrates the necessity of gradually increasing the desensitizing dose of the protein. Case 2, S. H. G., illustrates multiple sensitization and the specificity of proteins in the treatment of bronchial asthma, since during treatment with horse dandruff peptone alone there was little improvement, but during treatment with S. pyogenes aureus vaccines in conjunction with horse dandruff peptone, improvement was rapid and relief was obtained. This case like the preceding one illustrates the variety of symptoms associated with asthma. Case 3, L. T., who was relieved of asthma during treatment with horse dandruff coagulated protein,

shows nothing that the two preceding cases fail to show with the exception of the very early age of onset, which was since birth. Case 4, J. T. S., is of interest in that little or no improvement resulted from subcutaneous injections of horse serum, but relief from symptoms followed similar treatment with horse dandruff peptone, to which he was sensitive. Case 5, H. M., illustrates multiple sensitization and a difference between the various proteins in horse dandruff and in dog hair as measured by the cutaneous reaction. During treatment with S. pyogenes aureus vaccines the patient was free from asthma until he was exposed to horses and to dogs, each of which exposure was followed by asthma. Following three treatments with horse dandruff alkali meta-protein the patient remained free from all symptoms for thirteen weeks, when asthma followed exposure to horses; a previous exposure to horses during this time provoked cough, but no asthma. This patient, like a former one, S. H. G., shows the necessity in some cases of multiple treatment. Case 6, C. F. J., also illustrates multiple sensitization, the necessity of multiple treatment, and the effect of treatment with various proteins on the cutaneous reaction with these proteins. Treatment with one of the proteins in horse dandruff diminished the positivenesss of the cutaneous reaction for all of the proteins in horse dandruff, but this treatment had no effect on the skin reactions with the proteins found in cat and dog hair or in wheat. Treatment with one of the proteins in wheat reduced the positiveness of the cutaneous reaction with all the proteins in wheat. The remaining three cases, 7, 8, and 9, showed nothing additional to what the preceding cases have shown.

In Group II. three patients are presented, all of whom were sensitive to and treated with the proteins found in cat hair. Case 10, G. V. Y., is of interest in that the patient had indigestion with attacks of asthma, that the cutaneous reaction was positive with both cat hair proteins in a very high dilution, namely, 1–1,000,000, and that it illustrates the importance of gradually increasing the desensitizing dose of the

protein. The cutaneous reaction and the treatment in this case show that there is a difference between the alkali meta-protein derived from cat hair and that derived from horse dandruff. Treatment with the alkali meta-protein from cat hair relieved the patient of asthma and other symptoms and reduced the positiveness of the cutaneous reaction with both cat hair proteins one hundred-fold. Case 11, M. D., illustrates the association of asthma with menstruation, multiple sensitization, and a difference between the proteins of the same chemical nature, although derived from different sources. Improvement followed treatment with cat hair alkali meta-protein, but since the patient had much bronchitis, an autogenous sputum vaccine was given in conjunction with the cat hair protein; since this combination of treatment the patient has been free from cough and asthma even through her menstrual periods. Case 12, P. D., illustrates multiple sensitization and multiple treatment before relief was obtained; since an autogenous vaccine of the patient's sputum gave relief from symptoms, asthma in this case would seem to be caused by bronchitis at the present time, and the fact that he was sensitive to proteins may have been the primary cause at the onset of asthma.

In Group III. are presented eleven patients who were sensitive to and treated with the wheat proteins. Case 13, D. A. G., illustrates multiple sensitization and the specificity of proteins in the treatment of bronchial asthma, the very early onset of eczema, bronchitis and asthma, and the relief from symptoms while the patient was on a wheat-free diet in conjunction with subcutaneous injections of whole wheat protein. Case 14, C. K., was sensitive to only one of the wheat proteins, namely, natural proteose; little improvement resulted from a wheat-free diet, but relief from symptoms followed subcutaneous injections of the natural proteose, even while the patient was on a wheat-free diet. Case 15, C. N. E., in addition to what Case C 13, D. A. G., brought out, showed the variety of symptoms associated with attacks of asthma, and the marked improvement which resulted from a

wheat-free diet and from subcutaneous injections with the wheat proteins. This treatment rendered the cutaneous reactions negative with the wheat proteins, but it did not diminish the reactions with the proteins found in horse dandruff or in cat hair. Case 16, F. A., illustrates the early age of onset of asthma, multiple sensitization, and relief from symptoms while on a wheat-free diet, although her tolerance for small subcutaneous doses of wheat proteins was not much increased by these injections. Case 17, G. B., is practically identical to the preceding case. Case 18, G. MacD., illustrates the late onset in life of asthma, due to a few of the wheat proteins and the relief from symptoms while on a wheat-free diet in conjunction with subcutaneous injections of the wheat proteins. Case 19, M. S., illustrates the frequent necessity of vaccine treatment in conjunction with injections of wheat protein. Cases 20, W. P. H., and 21, J. D., illustrate extreme sensitiveness to the wheat proteins, the intolerance for small amounts of wheat protein when injected subcutaneously, and the relief from symptoms while on a wheat-free diet. The latter case is very interesting in that the patient could inhale wheat, eat wheat, or be subcutaneously injected with wheat without having asthma, but any combination of either two of these methods of taking wheat resulted in asthma. Cases 22, G. B., and 23, F. G., although sensitive to wheat proteins were not improved either by a wheat-free diet or by subcutaneous injections of the wheat proteins, thus eliminating wheat as the cause of asthma at the present time; since vaccines seem to give some relief these patients probably had more chronic bronchitis than asthma. Possibly the wheat proteins were the primary cause of asthma in these cases and the fact that the serum of these patients gave positive complement fixation reactions, using the wheat proteins as antigens, may explain why these patients have no asthma from the wheat proteins; these serum anti-bodies may be a protection.

In Group IV. are presented six patients who were sensitive to miscellaneous proteins. Case 24, H. T., illustrates

multiple sensitization and relief from symptoms when these proteins were avoided. Case 25, A. C., is similar to the preceding one in that both patients were sensitive to chicken meat. Case 26, W. M. O'D., was sensitive to casein, potato, and the protein of S. pyogenes albus, and although improvement followed a diet free from milk and potato, treatment with vaccines in conjunction with this restricted diet was followed by almost freedom from asthma. Case 27, A. G. C., who was sensitive to flaxseed protein, is presented to illustrate the combination of urticaria, asthma, and gastro-intestinal symptoms immediately following the subcutaneous injection of a very minute amount of flaxseed protein. Case 28, A. A. D., illustrates the effect of tobacco smoke on a patient with bronchial asthma. Case 29, P. J. F., is presented in order to illustrate a very hypersensitive skin and the deception of the cutaneous reaction in an occasional patient.

In Group V. are presented eight patients who were sensitive to the protein of S. pyogenes aureus and who were treated with vaccines of this organism. Case 30, M. L. S., was relieved of asthma during treatment with S. pyogenes aureus vaccine and remained free from symptoms for five months after treatment was discontinued; the patient then had a relapse of asthma, which was again relieved by similar treatment, and she has continued free from symptoms the remainder of the winter. Case 31, E. O'B., was not improved by treatment with a diphtheroid vaccine, but was relieved by S. pyogenes aureus vaccines, thus showing a specificity of bacteria in the treatment of bronchial asthma. Case 32, R. G. V., who was sensitive to the proteins of both S. pyogenes aureus and a diphtheroid organism, was relieved of all symptoms by treatment with vaccines of both organisms. Case 33, M. M., was greatly benefited for four months during treatment with S. pyogenes aureus vaccines, but when S. pyogenes albus vaccine was substituted the patient relapsed to her former condition, thus showing a specificity in bacteria in the cause and treatment of bronchial asthma.

Case 34, G., and Cases 35, A. P. J., and 36, R. B., were either relieved of asthma or were greatly improved by S. pyogenes aureus vaccines. Case 37, W. C. T., whose asthma was complicated by several chronic diseases, was not improved of asthma by a diphtheroid vaccine or by hospital treatment, but following S. pyogenes aureus vaccines he was relieved of asthma, thus in this case asthma and the other chronic diseases would seem to be of independent origin. The serum of five of these patients agglutinated strains of S. pyogenes aureus before vaccines were given, but after several vaccine treatments the serum of each case failed to agglutinate these organisms. The majority of these patients showed a variety of symptoms associated with asthma and all were relieved when the asthma was relieved.

In Group VI. are presented three patients who were sensitive to the protein of S. pyogenes albus and who were treated with vaccines of this organism. Case 38, A. A. B., who is a physician, illustrates the specificity of bacteria in the cause and treatment of bronchial asthma. He concluded that small, frequently repeated doses of vaccine gave better results than did larger and less frequently repeated doses, and that relief from asthma lasted for only a month. Case 39, F. S. E., was relieved of asthma for several months, then a relapse followed and he was a second time relieved of asthma for a time. Case 40, I. G., has been free from asthma all winter during treatment with S. pyogenes albus vaccines, with the exception of two nights when she ate honey; another patient has asthma after eating honey and both are sensitive to pollens which very likely contaminate the honey in the process of making by the bees.

In general, desensitization by subcutaneous injections of the proteins found in horse dandruff, cat and dog hair, and by treatment with S. pyogenes aureus and albus vaccines is very satisfactory and the patients are not only relieved of bronchial asthma, but they are relieved of the many other symptoms which are associated with bronchial asthma. At

present one cannot say how permanent the relief may be following treatment with the proteins found in horse dandruff and cat hair, but relief by vaccines seems to be temporary; it varies between four and six months after S. pyogenes aureus vaccines are discontinued and relief is less permanent after S. pyogenes albus vaccines are discontinued. Bronchial asthma which is caused by food proteins is relieved by omitting these proteins from the diet, but weekly subcutaneous injections of these proteins rarely increase the tolerance of the patient for them. The cutaneous reaction seems to bring out a difference between the proteins derived from the same source and supposedly closely related, as for instance, it separates the different proteins in horse dandruff, cat hair, and wheat. Subcutaneous injections, however, with one of these closely related proteins desensitizes against all of them, as for instance, treatment with one protein in horse dandruff desensitizes against all of the proteins in horse dandruff and the same holds true for the proteins in cat hair and in wheat. Treatment with horse dandruff proteins does not desensitize against cat hair or wheat proteins and this is true for treatment with cat hair or with wheat proteins. All of these facts seem to hold true for the various types of bacteria. There is certainly a specificity among proteins in the cause and treatment of bronchial asthma and treatment with any protein or bacteria will not relieve bronchial asthma.

Indigestion, dyspnea, colds and bronchitis, nervousness and irritability, all clear up under treatment with proper proteins without the use of drugs or hygienic measures. It is often difficult to tell whether colds and bronchitis are a part of the disease or whether they may be the cause or the effect. When these conditions are a part of the disease they clear up with protein treatment, but if this is not the case then these conditions themselves must be treated, and usually proper vaccines are sufficient. There seems to be two types of cold: one which is associated with patients who are sensitive to proteins and one which is very probably due to

bacterial infection. The former may be called an anaphylactic cold; it is of short duration, only a day or two, with no prodromal symptoms and it suddenly is well. The latter which seems to be of bacterial origin has prodromal symptoms for several days, it is very disagreeable and at a maximum for several days, and convalescence extends over many days. These same facts hold true for types of bronchitis. In other words, only patients who are sensitive to proteins have these short colds and attacks of bronchitis, but the infection type of colds and bronchitis occurs in both those who are sensitive to proteins and those who are not.

CONCLUSIONS.

Bronchial asthmatics who are sensitive to the proteins found in horse dandruff and in cat hair are relieved of attacks during a series of subcutaneous injections with these proteins. One cannot say as yet how long such relief will last after treatment is discontinued, but some cases have remained free from asthma as long as five and six months while treatment was continued.

Bronchial asthmatics who are sensitive to the proteins in S. pyogenes aureus and albus are relieved of attacks during treatment with vaccines of these organisms, and in the case of the former relief continues for four to six months after the vaccines are discontinued, but with the albus vaccines relief continues for a shorter time after they are discontinued. A second course of vaccines relieves a relapse of asthma quicker than did the first course.

Bronchial asthmatics who are sensitive to the food proteins are relieved of attacks and they remain free from asthma while such proteins are omitted from their diet. Subcutaneous injections of these food proteins at weekly intervals do not usually increase the patient's tolerance for these proteins and such treatment is of little or no value.

Patients who are sensitive only to closely related proteins are the simplest to treat and those who are sensitive to several types of proteins which are not closely related are

the most difficult to treat. This is because at first one cannot judge which protein is the cause of asthma at the present time, and so several proteins may have to be tried before the correct one is used. This multiple sensitization does not detract from the cutaneous reaction, since each of the proteins to which the patient is sensitive may have at some time been the real cause of asthma. We know that a patient who is sensitive to one protein is very likely, sooner or later, to become sensitive to others.

Patients with bronchial asthma associate attacks with cold air, dampness, changeable weather, winds, menstruation, indigestion, nervousness, irritability, colds, and bronchitis. After treatment with proper proteins these patients become tolerant to such conditions, so that they can be exposed to them without asthma and they become free from nervousness, irritability, and indigestion, without the use of drugs and hygienic measures. There seems to be two types of colds and bronchitis, one type is anaphylactic and relief or freedom from this type follows proper treatment with proteins, the other type seems to be caused by bacteria and frequently vaccine relieves and prevents these.

[I am greatly indebted to my assistant, Miss June Adkinson, for her valuable help and great interest in this work.]

REFERENCE.

1. Throughout this paper the dosage of the proteins has been given in minims, because tenths of a cubic centimeter were not always small enough fractions and we had no hyperdermic syringes graduated in one-hundredths. The syringes which were used allowed sixteen minims to the cubic centimeter, so that minims may be converted into tenths of a cubic centimeter if one desires.

A STUDY OF THE MICROÖRGANISMS OF DENTAL CARIES.*

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The literature upon the mycology of the mouth is voluminous, yet it contains no work upon dental caries that meets modern bacteriological requirements.

Miller[1] (1880–1890) has carried out the most extensive work done upon the subject of dental caries. He placed teeth in a fermenting mixture of bread and saliva. This he renewed from time to time. He succeeded after three months in producing artificial decay which "could not be told either microscopically or macroscopically from true caries." He demonstrated by zinc crystallization that such a fermenting mixture produced lactic acid. Upon this evidence he originated the lactic acid theory of caries. He strengthened his theory by presenting histological sections of decaying teeth. The bacteria that have the property of fermenting sugar are numerous. Miller had no thought of bacterial specificity in this action. He described a few oral bacteria, such as Leptothrix innominata, B. buccalis maximus, Jodococcus vaginatus, Spirillum sputigenum, Spirocheta dentium, but he himself said, "his bacteriological study upon caries had not been sufficiently extensive or conclusive" to warrant being incorporated into his book. His work was done thirty years ago.

Karolyi, Arkovy,[2] Dobrzyniecki,[3] Siberth, Vignal,[4] Rodella,[5] Goadby,[6] Choquet,[7] and others[8, 9, 10, 11, 12] have either advanced theories based upon bacterial activities, or have carried out bacteriological studies upon the subject of dental caries.

Among these writers Goadby[6] (1902–1903) has done by far the most important work. He has studied the microorganisms of the superficial and the deep layers of decay, and classifies these bacteria as acid formers and liquefiers. He finds that the deeper layers of caries contain a smaller

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variety of bacteria than do the superficial layers. In the deep layers of caries in twenty cases he finds constantly Streptococcus brevis, B. necrodentalis, and nothing else. In other work he adds to these Staphylococcus aureus. In the superficial decay he finds B. mesentericus, B. furvus, B. plexiformis, both Staphylococcus aureus and albus, Sarcinæ, and some other forms. He lays particular emphasis on the streptococcus and staphylococcus in connection with caries. Dobrzyniecki[3] (1902–1903) agrees with Goadby in his findings and in his opinions.

Choquet[7] (1900) isolated five organisms from decay beneath fillings. These were not familiar to other workers of his time.

Other students give conclusions concerning the process of caries based solely upon smear examinations; still others upon cultural studies in which the source of the specimens studied has been the saliva or a mixture of the saliva, tongue scrapings, and tooth scrapings — material remote from the actual carious process.

Kleigler[13] (1915) in his work upon oral microörganisms has made bacterial counts of the material collected from between the teeth, on tooth surfaces, and from carious teeth. He also has described many types of oral microörganisms. In caries he believes that the oral flora changes its character from a streptococcal to an acid-producing rod and a thread flora. He believes that in caries there is a large numerical increase in the oral microörganisms, but that the types actually concerned in the carious process are relatively few.

He describes B. mesentericus, B. putrificus, B. acidophilus (Moro), a non-pleomorphic beaded bacillus, a leptothrix which he believes he has cultivated, and Cladothrix placoides. He says that Goadby's necrodentalis conforms in most particulars to the acid-producing bacteria of Moro, Finkelstein, Rodella, Mereschkowsky, Kendall, and others.

It is evident from this review of the work upon caries that there is no agreement in the bacteriological findings of the various workers.

We have endeavored to study the bacteriology of dental caries more thoroughly and more in detail than has been done heretofore. The eighteen hundred children weekly treated for defective teeth at this institution afford at once an example of the need of such a study and an exceptional opportunity for it.

In order to arrive at something definitely conclusive in this work we have studied closely a larger number of cases than have previously been used. In order to be sure that we had under investigation the proper flora we have taken our material for culture from cases where caries is actively progressant. Caries is distinctly a disease of childhood, therefore we have confined our work to children.

For the same reason the sixth and twelfth year molars were selected. These are the teeth most subject to decay; they also furnish more material for study.

Inasmuch as pulp involvement complicates matters by introducing a different type of flora, care was used to avoid including such cases in our statistics. That uniform conditions might be assured, the children selected were of approximately the same age — that is, from ten to fourteen years.

The decay was studied under three different conditions:

I. Because the open carious tooth cavity is exposed to every kind of bacterial contamination, and because this adds confusion to the investigation, an attempt was made to eliminate the incidental invaders. A unique procedure was adopted for this purpose. Fillings were inserted over the bacterial carious mass, care being exercised not to injure this flora in any way. It seemed probable that the extraneous flora would die off, while the flora that had the best hold upon life in this environment would survive. After a period of from six weeks to three months the fillings were removed and the underlying carious dentine cultured.

II. The second condition under which the decay was studied was for the same purpose as the first. Fillings having slight antiseptic properties were placed over the carious tooth substance, but left for a much shorter period. Here we believed that the bacteria that would most readily yield to

the effect of the antiseptic would also give way to the more sturdy and vigorous flora intimately associated with caries. In thirty-seven cases, twenty-one had one member of the group, nine had two members, seven had three members.

III. The third manner of studying the carious tooth material consisted of a bacterial examination of the open carious tooth.

In all three procedures the teeth were isolated by the rubber dam, painted with iodine, and the material for study removed with sterile instruments and cultured.

The decayed material was removed in three layers: a superficial, a middle, and a deep layer, and later, from the deep dentine that appeared unaffected. Cultures were made from each stratum upon agar, glucose-agar, bouillon, and blood serum. These were grown aerobically and anaerobically.

It was seen that we were dealing with comparatively few organisms, and it was attempted to obtain these in pure cultures. Their morphological characteristics were studied extensively and they were subjected to biochemical and fermentation tests. It was evident from our studies that the constant and predominating flora of dental caries was the closely allied group of microörganisms described by Moro, Tissier, and others. These were found in all the cases of decayed teeth examined and were the prevailing bacteria. In cases that had been under fillings for three months and under fillings with slight antiseptic properties for a shorter time no other microörganisms were found. In the open carious teeth, yeast, Micrococcus catarrhalis, Staphylococcus, Streptococcus, and other bacteria were occasionally found, but there was no regularity in their appearance. Some member of, or the entire Moro-Tissier group, was always found, frequently nothing else. This group is tolerant of higher degrees of acidity than the bacteria above mentioned. It overgrows them in laboratory media. It forms very large amounts of acid. These properties account for the fact that the flora of the open carious tooth is not as varied as might be expected.

Under the first conditions of our study which we have designated as Class I., twenty-seven cases were examined after inert fillings, that had been inserted over carious dentine, had been removed. These specimens were cultivated aerobically. Eight of these contained two members of the Moro-Tissier group, three contained three members, six contained one member. It was rare to find any other organisms present. Ten gave no growth. This does not signify that bacteria were absent from the decay, for the specimens were cultivated only aerobically, and as the growth in the tooth cavity had been under complete anaerobic conditions, without doubt the transition to full aerobiosis was too radical a step. Our other work supports this interpretation. We have never failed to find life in such cases when anaerobiosis was employed. It is admitted that this group grows best anaerobically. B. bifidus is held by some to be a strict anaerobe.

The essential point is that in these cases nothing else was present except members of the Moro-Tissier group.

In Class II., after the removal of fillings containing slight antiseptic properties, the cultured decay of the tooth gave similar results.

Class III. was particularly interesting, for it showed that the flora of the open carious cavity is not varied. On the other hand it is limited.

The acidific group is always present. In some cases nothing else is present and no other microörganisms appear with any regularity.

Of the eighteen cases studied, some member of the group is present in every one. Seven of the cases contain nothing else. Seven contained three members of the group, five contained two members, four contained one, and two contained four. Yeast appeared in the superficial layer in seven cases, Micrococcus catarrhalis was found in four cases, Streptococcus in three, and Staphylococcus in three.

It is readily seen from these statistics that the only constant flora in dental caries is included in the Moro-Tissier group. It is equally evident that this is the predominant flora. Nothing else was present in the cavities that had had

the extraneous flora excluded by fillings. Nothing else was present in 38.8 per cent of the open cavities. It is natural to expect this limitation in the types of the flora of dental caries when once its character is disclosed. For this group of microörganisms is known and its high acid-producing character is established. High acidity is not withstood by many organisms, so that the extraneous bacterial life does not here flourish. These microörganisms by their high acid-forming properties possess, in a greater degree than do others, the capacity for at least inaugurating the carious dental process. They form as we shall see further on more than fourteen per cent normal acid. They soften and produce results upon tooth sections that are similar to tooth decay in the mouth.

Before entering upon the description and classification of these microörganisms that we have found and studied in caries, some of the characteristics of this group may be mentioned. Moro,[14] Tissier,[15] Rodella,[5] Cahn, Passini, Herter,[16] Kendall,[17] Noguchi,[18] and others have done extensive work upon these bacteria and have shown them to be the prevailing flora of the nursling's intestinal tract. The majority of observers have found the aciduric bacteria localized in the large intestine, and only under exceptional circumstances or conditions do they occur in the higher levels of the alimentary tract. Weiss believes that they would appear in the "more acid portion of the tract." Under normal conditions then these microörganisms constitute the flora of the acid intestinal contents of the nursling. Their presence in dental caries has never been previously known.

The presence of B. acidophilus in the mouth has been noted, and Lewkowicz[19] mentions finding B. bifidus in the mouth of nurslings. But the presence of these microorganisms in all dental caries constituting the predominating flora is a matter of interest in view of what has been said and of the statement by Noguchi that "in spite of numerous attempts as yet no one has succeeded in tracing the source of B. bifidus outside of the intestinal tract." Why and how the group occurs in dental caries is not known.

It is natural to expect this Moro-Tissier group to have different characteristics in these two different parts of the alimentary canal, but in the respects of acid tolerance and high acid production they are very similar in both places.

Another thing that has been the subject of much study and discussion in connection with this group of microorganisms is their highly pleomorphic character. Moro continually mentions it. Noguchi has treated the subject in a most interesting manner in an article that gives an account of his study upon B. bifidus. He entitles his work, "The pleomorphism of B. bifidus communis." He says: "The relation between B. bifidus communis and certain other Gram-positive organisms of the feces such as B. acidophilus (Moro), Kopfchen-bacillus, B. tubercular-formis intestinalis (Jacobson), B. infantalis (Kendall) remains unsolved." At present it is difficult to say whether they are entirely different organisms, or whether they are different forms of one and the same organism. He was "able to show B. bifidus to be an anaerobic phase of an aerobic sporagenous organism belonging to the subtiloid group and closely resembling, especially morphologically and biologically, B. mesentericus fuscus."

He shows B. bifidus in forms and groupings similar to acidophilus. He shows V-shaped, Y-shaped, fishtail and bifurcating forms, streptococcal and staphylococcal forms, rods and spores. He has been able to modify these and to change B. bifidus by varying degrees of anaerobiosis and aerobiosis from one extreme form to the other.

Noguchi emphasizes the importance of this in interpreting bacterial findings. We have been able to find all these forms in dental caries. We have been able to change them into different and characteristic forms by prolonged cultural work. We have succeeded in producing a sporulating stage from a bifurcating form and in modifying the spore-forming stage to the rod form by changing the aerobic to anaerobic conditions.

The question of pleomorphism has not been confined to B. bifidus. B. acidophilus has also been studied and is

constantly termed "highly pleomorphic." Our studies seem to indicate that in the case of this microörganism the pleomorphism has been exaggerated.

The importance of taking this pleomorphism into consideration in the interpretation of bacteriological findings in the study of carious teeth is hard to overestimate. Indeed, the complete lack of attention it has received has obscured the whole of the bacteriological work heretofore done upon dental caries.

The organisms constantly found in dental caries, and belonging as we believe to the Moro-Tissier group because of their highly aciduric character, we have called: Bacillus acidophilus (Moro), Bacillus X, Bacillus M, Bacillus Y, B. bifidus.

Bacillus acidophilus of Moro as found in dental caries is a non-motile, non-pathogenic rod. When grown on agar it is short and thick and measures .75 x 2 to 3 microns. It is Gram-positive.

On glucose-agar the rods are longer and thinner and more distinctively arranged in groups with the individuals showing parallelism. They produce turbidity of the media. They are Gram-negative. On blood serum the rods measure .1 x 1 microns. They are Gram-negative, grouped as in glucose-agar.

Bacillus acidophilus grows best anaerobically when first isolated by means of acid broth. In milk it clots the lower portion first. In peptone water it produces no indol or ammonia. In broth it forms a heavy sediment with some turbidity. It forms no gas in sugar. It is a facultative anaerobe and should be transplanted every ten days. It is a high acid former. Most strains ferment saccharose, glucose, lactose, maltose, and raffinose. From seven to ten cubic centimeters of N/NaOH is required to neutralize one hundred cubic centimeters of the bouillon. It does not ferment lactose as readily. According to the studies we have made it is not as pleomorphic as has been supposed. The colonies are slightly raised, round, smooth, opaque, and white.

Bacillus X is somewhat pleomorphic. It is Gram-positive on agar. It most frequently appears as a long chain of short, thick rods, 5 x .5 microns, the chains often showing parallelism. Under certain conditions the individuals are considerably longer and do not occur in chains. Moreover, smears of the organism often show only masses of long, tangled, unbroken threads, occasionally having one long thickened end. On glucose-agar many of the individuals fail to retain the Gram stain. It is an anaerobe facultatively aerobic. It produces a high degree of acidity often requiring fourteen cubic centimeters of N/NaOH to neutralize one hundred cubic centimeters of the bouillon. It ferments glucose, saccharose, and levulose, but does not ferment lactose readily. It coagulates milk and does not form indol or ammonia.

On petri dishes its colonies are transparent, round, entire, slightly raised.

Bacillus M is a small, slightly curved rod, Gram-positive on agar, Gram-negative on glucose-agar and blood serum. In smears the organisms appear as bent individuals, in pairs, with concave sides facing each other, and in clusters, sometimes joined end to end and thus forming sectors of a circle. It possesses the same high, acid-forming properties as does Bacillus X. It grows best on glucose-agar, on which it forms small, round, convex, cream-white to brownish colonies, the pigment increasing with age.

Bacillus Y is a straight or slightly curved rod with rounded and sometimes tapering ends. It measures from one to two microns x .2 of a micron. The cells are arranged side by side. It is Gram-positive on agar and on glucose.

On blood serum it occurs in the form of rods three to six microns long, and as long, winding threads with a width of .5 of a micron. The ends are frequently thickened. Both rods and threads are Gram-negative and may contain one or more Gram-positive bodies.

This microörganism we have found but one or two times. We have reserved it for future study.

B. bifidus, as it appears in the carious tooth, is to be found

in many forms. It is found frequently in its bifurcating state with tapering or with thickened ends, in V and Y forms, in streptococcal forms, as masses of Gram-negative cocci, as straight rods, in crosses and as a spore-former. It is Gram-positive in young cultures. It grows aerobically and anaerobically, although according to Kendall it is a strict anaerobe. Noguchi shows it to have both anaerobic and aerobic phases. In contradistinction to his, the bifurcating form of our organism grows well aerobically after adaptation to artificial media. The colony is raised, white, entire, butyrous.

CONCLUSIONS.

1. The Moro-Tissier group of microörganisms is the constant and predominant flora of dental caries.

2. These closely allied organisms from dental caries present the same morphological features as do those isolated from the intestine of nurslings.

3. Their high acid-forming properties limit the character of the flora found in carious teeth.

4. They possess in a greater degree than do any other organisms, the attributes that are considered necessary for at least inaugurating the process of dental caries.

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DESCRIPTION OF PLATES XXXVIII.–XL.

(These are all anaerobic cultures. Figs. 15, 16, 17, and 18, Plate XL., are the same strain.)

PLATE XXXVIII., FIG. 1. — B. acidophilus. Forty-eight-hour culture on glucose-agar. Gram stain x 2,000.

FIG. 2. — B. acidophilus. Seventy-two-hour culture on agar. Gram stain x 2,000.

FIG. 3. — Bacillus M. Forty-eight-hour culture on glucose-agar. Gram stain x 2,000.

FIG. 4. — Bacillus M. Forty-eight-hour culture on agar. Gram stain x 2,000.

FIG. 5. — Bacillus Y. Seventy-four-hour culture on glucose-agar. Gram stain x 2,000.

FIG. 6. — Bacillus Y. Ninety-six-hour culture on blood serum. Gram stain x 2,000.

PLATE XXXIX., FIG. 7. — Bacillus X. Forty-eight-hour culture on blood serum. Gram stain x 2,000.

FIG. 8. — Bacillus X. Forty-eight-hour culture on glucose-agar. Gram stain x 2,000.

FIG. 9. — Bacillus X. Forty-eight-hour culture on agar. Gram stain x 2,000. Note variation in length of bacilli, also heavy thread forms.

FIG. 10. — Bacillus X. Same. Note tangle of threads and chains.

FIG. 11. — B. bifidus. Forty-eight-hour culture on agar. Gram stain x 2,000. Staphylococcal form.

FIG. 12. — B. bifidus. Culture on agar. Anaerobic two days at 37° C. Aerobic eight days at room temperature.

PLATE XL., FIG. 13. — B. bifidus. Culture on glucose-agar eighteen days. Gram stain x 2,000. Streptococcal form.

FIG. 14. — B. bifidus. Forty-eight-hour culture on blood serum. Gram stain x 2,000.

FIG. 15. — B. bifidus. Seventy-two-hour culture on agar. Gram stain x 2,000.

FIG. 16. — B. bifidus. Culture on agar. Seven days. Gram stain x 2,000. Rods and spores.

FIG. 17. — B. bifidus. Culture on glucose-agar. Seven days. Gram stains x 2,000. Rods.

FIG. 18. — B. bifidus. Culture on glucose-agar. Eleven days. Gram stain x 2,000. Rods and crosses.

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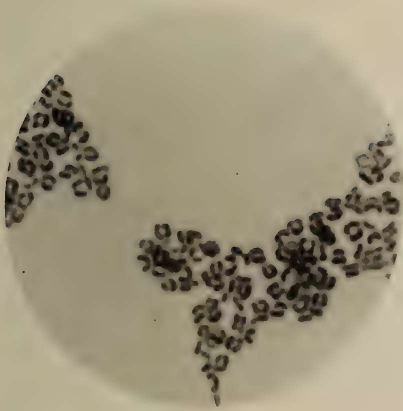

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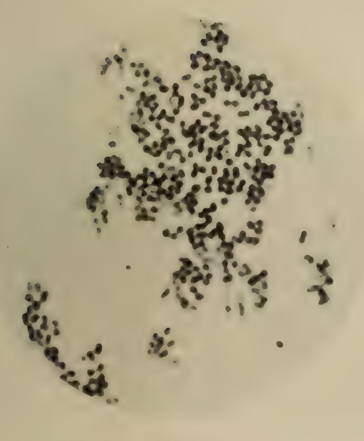

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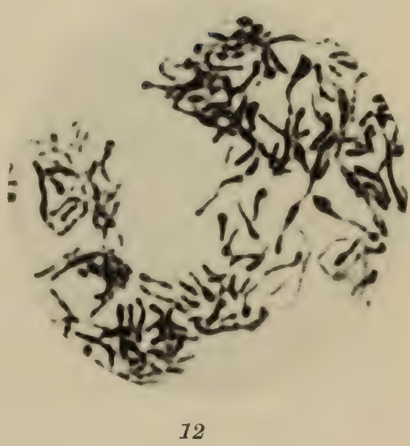

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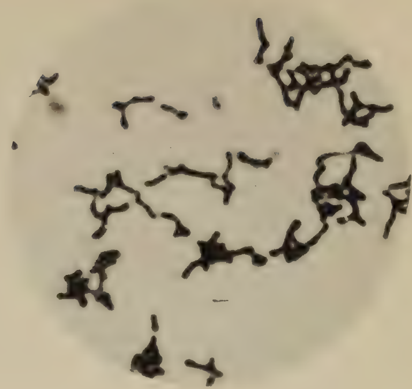

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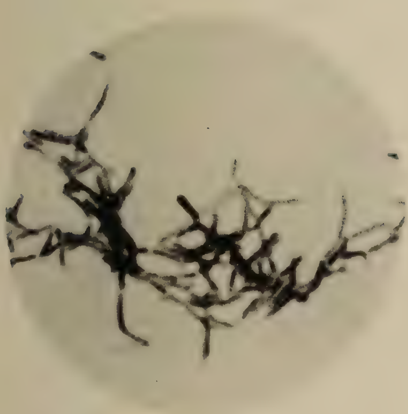

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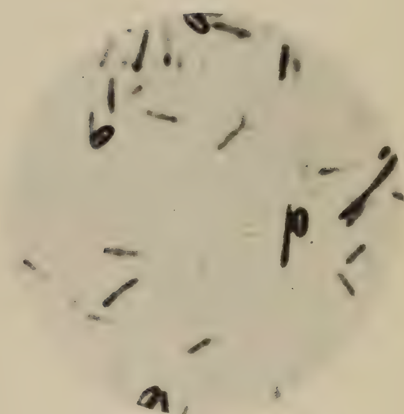

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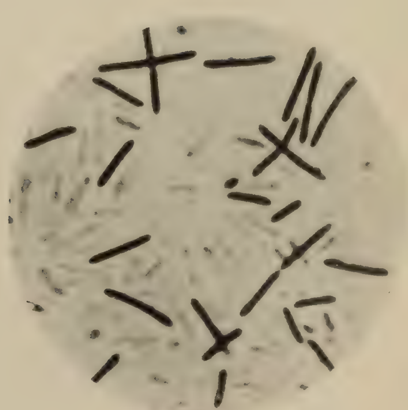

18

STUDIES IN CALCIFICATION BY THE USE OF VITAL DYES.*

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Introduction. — The specific vital staining of developing bone by the feeding of the dyestuff madder has been common knowledge for some hundreds of years, and it has also been known for a long time that the callus formed during repair of bone injuries is, in the madder-fed animal, stained like bone. From a survey of the madder literature it appears that, under the usual conditions of madder-feeding, the red coloration is confined to the part of the bone or callus laid down during the time that the dyestuff was present in the circulating blood; that it is due to the staining of the bone salts by the active principles of madder, alizarin, and purpurin, of which alizarin is much the more important; and that these dyestuffs are made effective by combination with calcium.

The fact that the staining phenomenon is dependent upon the concentration and precipitation of madderized calcium salts suggested the experiment of feeding madder to animals in whose tissues pathological concretions were forming, for it was anticipated that these deposits would stain similarly to developing bone. Furthermore, since the method of madder-feeding has proved of service in analyzing the growth of bone by providing a means of differentially staining the part deposited during any given period, it was thought that the growth of non-osseous accumulations of calcium salts might also be studied in this way. Accordingly, the following investigations were carried out:

Experiments and observations. — The work was done principally with the rat (the albino, for the most part), which for the purpose of the experiments is ideal, since it eats food

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containing madder very readily, thrives upon this diet, and gives no evidence of being deleteriously affected. It is only necessary that a small quantity of the dyestuff (one to five grams for each adult animal) be thoroughly mixed with the food, and fed once daily. The stain rapidly appears in the calcium-salt deposit. In the bones of a young rat receiving madder in solid food as well as in the mother's milk distinct staining has been found after only twenty-four hours' feeding.

The splendid rat colony of the Wistar Institute of Anatomy and Biology, Philadelphia, supplied most of the material, and the regular rat dietary of selected table-scrap, which has given such satisfactory results at the Wistar, was used.

After killing, the material was fixed in ten per cent neutral formalin, washed, and dehydrated. Gross specimens, or thick freehand sections of these, were cleared by the methods of Spalteholz or Schultze (Macklin[1]). Thin sections of the material were also cut in the usual manner.

Vital staining of calcifying areas in the kidney. — Calcification may be induced experimentally in a number of ways, one of the easiest being by permanent unilateral ligation of the renal vessels. Von Werra[2] and others have shown that calcification takes place in the kidney of the rabbit in a short time following this procedure, and Wells, Holmes, and Henry[3] believe that this is a typical pathological calcification. If the operation is done carefully and aseptically the animal soon recovers and shows no ill effects.

This experiment was performed in two cases in rats aged six months and one year, the ureter being included with the renal artery and vein. In both animals the central area of the ligated kidney became the seat of a process of calcification, the organ shrinking very markedly in size until it was but a mere fraction of the volume of its partner, which meanwhile underwent compensatory hypertrophy.

In one of these cases — a black rat six months old — after fifty days' normal existence following the operation, madder-feeding was carried on for ten days, and the kidney

then examined. In the gross it was very small, shrunken, and firm, with a light-colored and granular exterior, and was strongly bound down by fibrous adhesions to the posterior abdominal wall, spleen, and stomach. Through these adhesions a collateral circulation had been established. Upon cutting into one end of the kidney the interior was found to be pulpy, and in this pulp were numerous hard white or pink grains of different sizes. These were masses of calcium-salt compounds which had been deposited in the sixty days since operation, and the pink coloration was due to the effect of the madder-feeding.

After fixation and hardening in alcohol the kidney was cut across, this operation being accompanied by a gritty feeling. The cut surface presented numerous white masses in the central region of the kidney remnant, and, as well, many similar masses stained more or less strongly red. The color was similar to that of madder bones.

Thick freehand sections, cleared according to the method of Spalteholz, show the stained areas of calcium salts very clearly indeed (Fig. 1). Under the binocular microscope many irregular white nodules are seen, some being encrusted or mottled with red granulations, while other granules are completely stained. Sometimes a more or less complete shell of stained material will enclose an unstained concrement. The intensity of staining varies from light pink to deep red.

Thin sections cut from imbedded material show the outer zone of the calcified kidney made up principally of fibrous tissue, which has largely replaced the original epithelium. The entire central portion of the kidney is filled with masses of calcium-salt concretions, surrounded by leucocytes and fibrous tissue. Here and there red areas appear in the calcareous material, corresponding to the red-stained nodules and granules seen in the gross specimens. The gradation in staining intensity is strikingly shown.

No ossification was observed. Calcification involves all the tissues, interstitial and parenchymatous, as in Kidney

VII. of Wells, Holmes, and Henry[3] (page 384). The kidney parenchyma has almost completely disappeared, being represented only by scattered degenerate tubule remnants. Hence the calcium deposit bears no relationship to the urine, but must have come from the blood.

It appears from these findings that the salts of calcium in the calcified kidney from the madder-fed animal are stained, but that the staining does not involve the entire deposit — at least when the feeding is of short duration. Only certain parts take the dye. It has been noted that growing bone shows a similar staining reaction, where the young animal has been fed madder for but a short time, and, as has been mentioned, the explanation for this phenomenon has been advanced that the stained areas of bone were formed during the period of madder-feeding, calcium salts which were precipitated during this time being infiltrated with the dyestuff. If the same explanation holds for the calcium-salt deposits of the kidney described, and there is every reason to believe that it does hold, then those areas which are stained may be interpreted as having been formed during the last ten days of the lifetime of the animal, *i.e.*, during the time that the dyes of madder were being precipitated into the calcifying tissue. Since the stained areas are scattered and irregular, it appears that the deposition of the salts of calcium has been sporadic. That the nodules, at first small granules, grow by accretion is evident from the fact that some are found with an outer shell of stained material. These nodules finally become more or less homogeneous masses, as shown by the sections.

This description agrees with that of ossification by Pacchioni,[4] who found that the bone salts were deposited at first as granules which later developed into homogeneous masses.

It has been mentioned that the shade of red was of varying intensity, some regions being a mere light pink, while others were of quite a deep red. Similar findings have been described in madder-stained developing bone and the same explanation which has been advanced for the phenomenon in the latter tissue (Gottlieb[5]) may be applied, viz., that the

lighter pink areas represent those parts where the precipitation of calcium salts was almost complete when the madder-feeding was begun, and hence where but little of the dyestuff was deposited, whereas the deeper red parts mark out places where the precipitated salts have all, or almost all, come down during the period of madder-feeding.

It is possible, by this means, to analyze the growth of such calcium-salt deposits by feeding madder in a series of cases for short periods, at varying intervals after the operations. It would be also possible to return to the regular diet, following a period of madder-feeding, and in this case we may predict that the nodules of red would be encrusted with unstained calcium salts in the same manner that madder-stained spicules of bone are covered, under similar conditions.

Vital staining of the calcifying crystalline lens. — Cataract, or degeneration of the lens and deposition in its substance of opaque precipitates largely composed of salts of calcium is occasionally found in the young albino rat. A rat six weeks old, showing this condition in both eyes, was presented to me by Dr. Stotsenburg, of the Wistar Institute, with the suggestion that it be put upon a course of madder-feeding. The eyes were an opaque, grayish white, and the animal was quite blind.

After three days of feeding with madder the eyes had become distinctly pink. This diet was continued for six days, and the animal was immediately killed. In the gross the lenses were pink, somewhat irregular and shrunken. After fixation and clearing it was found by inspection with the binocular microscope (Fig. 2) that the staining was of varied intensity and was confined to discrete granular masses of different sizes. Some of the larger masses resembled crystals in their outline, but most were irregular. No colored encrustations around uncolored masses, like those of the calcified kidney, were found in the lens. As a rule the stained masses were situated at the periphery. In the

center of the lens was to be seen a nucleus composed of lens fibers which were not yet calcified.

Sections were cut from one of the lenses after imbedding, and were stained in different ways. The capsule was irregular in outline (Fig. 3). Through almost the entire area calcified degenerate tissue was seen, some regions being almost homogeneous in structure, though most of the calcareous material was granular. Here and there remains of broken-down lens fibers were found, especially toward the center of the mass, where an aureola of tattered and frayed-out remnants enclosed the lens nucleus. With Mallory's connective-tissue stain the latter takes the fuchsin, and appears red, fading into a dull bluish purple peripherally.

Here and there, principally near the surface, can be seen the faintly-stained areas where the dye has been deposited. These areas are of a looser texture than the surrounding more compact substance, and in the cleared unstained sections are seen to be composed of abundant refractile granules which are stained pink. The process of calcification is evidently not so advanced here as in the parts nearby. The stained areas stand as a proof that calcification is still going on.

The condition in the lens resembles very much that in the kidney, the madder-coloration being the same. Perhaps the most outstanding similarity is the irregular nature of the staining, for, in the lens as in the kidney, most of the calcareous material is without color, the latter being found only in certain isolated areas, usually small and granular in character.

Burge[6] has shown that in cataract there is a decrease in the percentage of potassium and an increase in that of sodium, calcium, and, in the lenses obtained in the United States, of magnesium. The madder-dye compounds of the latter two elements are insoluble, and, during the precipitation of the other insoluble and opaque salts of calcium and magnesium they also are precipitated and thus stain the cataract.

The examination of these specimens shows that this calcification of the lens takes place in special more or less sharply defined areas, rather than throughout the lens substance, at any one time, and by the enlargement and interfusion of these nodules, and the formation of new ones, the substance of the lens is gradually resolved into a mass of calcium salt.

It appears that the central part of the lens is less liable to this calcification process, for here the fibers remain intact. The reason for this is not clear, but it may be that it is due to the greater difficulty in transporting to this part of the lens-mass the necessary materials for calcification. That calcification in the stage examined is proceeding more rapidly at the periphery of the lens than anywhere else in its substance is shown graphically by reference to the position of the stained areas (Fig. 3).

It may be well to mention that the normal lenses of the rat do not stain with madder, even after five months' continuous feeding.

Vital staining in calcifying cartilage. — In the course of some experiments on the prolonged feeding of rats with madder it was discovered that the characteristic staining was found in certain areas of the cartilage. Several litters of rats were fed daily with madder from birth for extended periods. The dyestuff was at first given to the sucking animals by feeding it to the mother, the milk being in this way charged with it.

Distinct staining was noted at the end of three months, and this coloration was even more marked after more prolonged feeding. Specimens cleared by the Spalteholz method show that the cartilage is not stained in its entirety, but that the stained portion is represented by a lace-like core, surrounded by colorless hyaline cartilage (Fig. 4). Cartilages of the trachea, cricoid, xyphoid process, and ribs were found to be distinctly stained, but in no case completely.

Sections were cut and show the stained area in the central part of the cartilage, surrounded by normal cartilage cells (Fig. 5). Its structure is reticular and it is not so densely

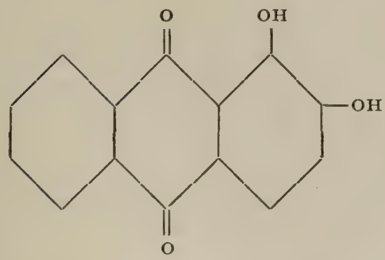
stained as bone, since deposition of calcium salt in this tissue is not so rapid nor abundant as in bone. This latter circumstance also probably accounts for the staining in calcified cartilage not being visible until after prolonged feeding with madder.

Weak staining of the trabeculæ of calcified cartilage in the epiphyses of developing bone was also observed. The staining was accomplished in less time here, since the circulation is much better. Thus the areas of calcium-containing cartilage are differentially marked out.

It is evident that madder will vitally stain pathological and non-osseous physiological accumulations of calcium salts as well as the calcium salts of developing bone. This confirms, in some measure at least, the finding of Gottlieb[5] that "der Krapp und in ihm das Alizarin ist ein vitaler Farbstoff für die kalkhaltigen Gewebe," and suggests that all pathological calcific deposits in the living organism will stain with madder; but it seems to be necessary that to ensure such staining, by ordinary methods of madder-feeding, the salts entering into the formation of these deposits should be exposed to the influence of the stain during the time when they are being laid down.

Discussion. — Madder, the dried and ground root of the plant erythrodanum or rubia tinctorum, has been used in the dyeing industry from time immemorial, and it was the first "vital dye" to be described. In attempting to explain its action in staining calcareous deposits it is necessary to consider its active coloring principles, alizarin and purpurin, and to refer to the literature on the madder-staining of growing bone. Gottlieb[5] has reviewed this literature carefully, and from a consideration of his paper, and other sources, the following facts are brought out:

Alizarin, or 1 : 2 dihydroxyanthraquinone, is a derivative of anthracene with the formula $C_{14}H_6O_2(OH)_2$ (Thorpe[7]), graphically represented thus (Houben,[8] page 966):



According to Gottlieb,[5] it was recognized by Lieberkühn in 1874 as the coloring agent of madder responsible for the vital staining of growing bone. Though almost insoluble in water, Perkin and Kipping[9] (page 521) state that "with aqueous solutions of the alkalis it forms metallic derivatives of the type $C_6H_4\langle{}^{CO}_{CO}\rangle C_6H_2(OM)_2$, which are soluble in water, yielding intensely purple solutions." With the alkaline earths it forms insoluble precipitates or lakes, as the purple precipitate calcium-alizarate, $CaC_{14}H_6O_4$ (Thorpe[7]).

In the ground root of madder, alizarin exists as a glucoside, known as ruberythric acid or rubian, $C_{26}H_{28}O_{14}$ (Perkin and Kipping,[9] page 520). This material is not a staining agent until it is broken up, which is easily accomplished through the action of an enzyme, contained in madder-root, and also in other ways, as by weak acids, *e.g.*, gastric juice.

The probable course of events in the staining of calcareous deposits is as follows: alizarin, split off from its sugar during gastric digestion, if not before, passes into the duodenum, and enters an alkaline medium. It is here rendered soluble, appearing no doubt largely as disodium alizarate (according to Gottlieb some may be combined in the digestive tract with calcium) and is speedily absorbed into the blood stream.

To assist in understanding the subsequent events in the transportation and deposition of alizarin, with the resultant staining of the calcium-salt mass, it may be of assistance to refer to some of the current notions as to calcification and ossification. Wells,[10] assisted by the ideas of Hofmeister, Barillé, and others, has set forth a very attractive hypothesis

to explain these processes. He assumes that calcium phosphate and calcium carbonate, the principal salts of bone and calcific deposits, are held in solution by the colloids of the blood plasma, and that their solubility is enhanced by the CO_2 of the blood. When this colloidal solution comes into contact with a hyaline material, such as the osteoid-tissue of developing bone, or the degenerate matrix of calcifying tissue, there is assumed to be an adsorption of the calcium salts into this matrix. If the CO_2 be now diminished, there follows as a consequence a precipitation of the salts, and these processes of adsorption and precipitation may be conceived of as proceeding rhythmically until the hyaline matrix is saturated with the calcium salts. Since the proportion of the constituent salts in the blood is determined by their relative solubility in the plasma medium, it follows that they will be precipitated in bone and in calcific deposits in the same relative proportion, and this supposition has been verified by experiments, for analyses of bone and of calcareous deposits have revealed the fact that not only are the salts the same, but their relative proportions are also, in general, the same.

These facts have led Wells and others to look upon calcification and ossification as expressions of the same general physico-chemical process, the only essential difference being that in ossification the processes involved are under cellular control, whereas in calcification they are not. That calcification of the lens, for instance, is largely a physical process occurring after degeneration of the protoplasm, which renders it an available dumping ground for the insoluble salts of the body, is realized by the following extract from Burge[6]:

> "In cataract, the lens loses the characteristic ash content of a living tissue and approaches the composition of the liquids of the body to the extent that its content in potassium is diminished and its content in sodium and calcium is increased."

These ideas may now be applied to the elucidation of alizarin metabolism. Though some of the alizarin in the blood may be carried as the soluble sodium compound, and as such be readily excreted by the renal epithelium, it is

quite possible that a great deal of the dyestuff is combined in the blood with calcium, as Gottlieb[5] holds, and in this condition we may believe it to be held in solution by the colloids and CO_2 in the same manner that the salts, calcium phosphate and calcium carbonate, are thought to be held.

In the course of its circulation through the tissues the alizarin compound may now be assumed to enter a region where calcium is concentrated, as in a growing bone or pathological concrement. If it has not already done so it now combines with calcium to form the lake, calcium alizarate, and its subsequent behavior is similar to that of the phosphoric and carbonic acid compounds of calcium, that is to say, it is probably adsorbed by the hyaline matrix and precipitated therein. Calcium alizarate is thus built into the new bone or calcareous deposit with the carbonate and phosphate of calcium, and the manner of its deposition is probably similar to that of these precipitates.

Since the red color of the deposit is due to the mixture of the insoluble salts of alizarin with the insoluble salts of phosphoric and carbonic acids, as these are being precipitated, there is no characteristic staining of the calcium salts already laid down, provided alizarin is not in excess. It is possible, however, that if the body-fluids be overloaded with alizarin all of the calcium-salt deposit would be stained. Such a staining is described by Gottlieb[5] for bone, but here the doses of dyestuff were very excessive and were given parenterally. Then, too, the stain was different, being violet, and not red, and the relationship between the dyestuff and the bone in these cases is said to be different. However, where the concentration of alizarin is so slight as it is in the blood of madder-fed animals, where feeding has not been forced, there is very little, if any, staining of the calcium salts already laid down.

The explanation of this differential staining may be, as Gottlieb[5] suggests for the same phenomenon in bone, that all the alizarin in the blood is combined with calcium, so that there is no dyestuff available for combination with the bone salts already deposited.

The intensity of staining of any given area depends largely upon the measure of its capacity for adsorption of calcium salts. For instance, if it contains little or no salts to begin with its capacity will be great, and it will stain densely, whereas if it is almost or quite saturated with them it will stain faintly or not at all. Again, one type of matrix may be capable of adsorbing more salts than another. Thus we find all grades of coloration from light pink to deep red.

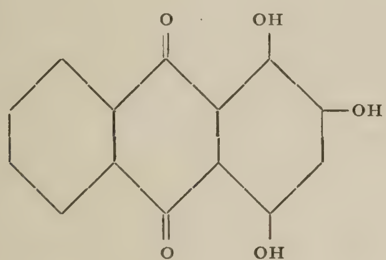
Gottlieb [5] assumes that, in the case of madder bones, a small proportion of the alizarin is deposited as a true lake by uniting with an element which acts as a mordant, in addition to the calcium. Such a compound would be resistant to the action of dilute acids. Whether or not such a reaction occurs in calcareous deposits other than bone must remain, for the present, an open question.

It has been mentioned that ossification and calcification have been regarded as expressions of the same physicochemical processes, the identical salts being in each instance deposited into a similar matrix, and in the same proportions (Wells [11, 10]). It would seem that, in the results of the above experiments, we have further evidence pointing to a fundamental identity of these processes, for not only is the same color taken by growing bone and developing calcific deposits exposed to the action of madder dyes in the circulating blood, but, under the conditions of the experiments, this color seems to be limited to the salts deposited during the period of administration of the dye, and its shade varies directly in proportion to the extent of the calcium-salt precipitation.

The same staining effects on calcific deposits would doubtless be obtained with alizarin alone as with madder. Gottlieb [5] has mentioned that he was able to stain the bones and teeth of the rat by feeding it alizarin, and I have verified this observation in my own experiments. The slightly different color of the bones from the animal on the pure alizarin diet as compared with those of the madder-fed animal is accounted for by the presence in madder of the dyestuff purpurin,

which, since it is a brighter red, somewhat modifies the shade (Gottlieb [5]).

What has been said regarding alizarin-staining applies also to that of purpurin, though the latter dyestuff is much less important, due to its being present in madder in much smaller quantity than alizarin. Purpurin, 1 : 2 : 4 trihydroxy-anthraquinone, is also a derivative of anthracene, and its formula, $C_{14}H_5O_2(OH)_3$, may be expressed thus (Houben,[8] page 971):



It forms insoluble salts with the alkaline earths and behaves in ossification or calcification similarly to alizarin.

Magnesium, as well as calcium, plays a part in bone or calcific deposit formation, and combines with alizarin and purpurin in the same manner as calcium to form insoluble lakes. Though of relatively less quantitative importance it may be considered as entering to a slight extent into the production of the madder-staining phenomenon.

SUMMARY.

The following conclusions, based upon the results of the foregoing findings, may be drawn:

Non-osseous calcareous deposits, such as calcific granules in the permanently ligated kidney and in the lens in cataract, are distinctly and specifically stained by feeding madder. When the dyestuff was fed during only a part of the period when the concretion was forming the stain is found only in parts of the concretion, and these stained areas probably represent the masses which were deposited during the time

that the dyestuff was being fed. In these respects, and in the fact that the intensity of staining differs in different areas in direct proportion to the extent of the deposition, during the period of madder-feeding, of calcium salts, the phenomenon is analogous to that which obtains in developing bone of the madder-fed animal. Thus the conception of a closely similar or identical physico-chemical process in the formation of developing bone and of pathological calcific deposits is strengthened.

Not only is calcified cartilage, formed in the development of "cartilage bone," vitally stained by madder-feeding, but the portions of any cartilage which contain salts of calcium are vitally stained with the dyes of madder if feeding is prolonged. This staining was found in rats fed continuously for three months or longer.

[Finally, I desire to express my cordial thanks to Miss Madge DeG. Thurlow, who kindly assisted with the operations; to Drs. Greenman and Donaldson of the Wistar Institute for the facilities of the laboratory; and to Dr. J. M. Stotsenburg for his untiring efforts in obtaining material.]

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DESCRIPTION OF PLATE XLI.

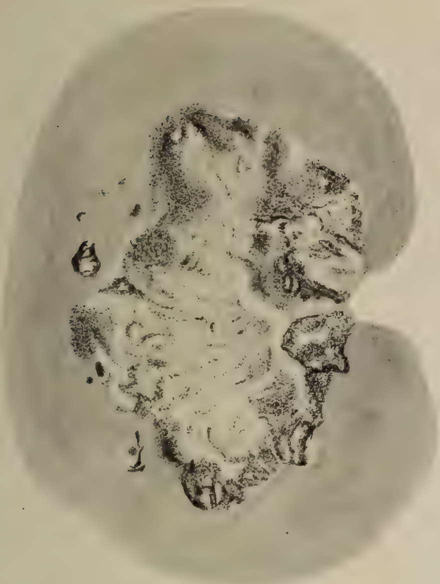
Plate XLI., Fig. 1. — Drawing of a cross-section from a rat's kidney, the vessels of which had been ligated permanently sixty days before animal was killed. During the last ten days of life madder was fed. Free-hand section, about one-third millimeter thick, cleared in oil of wintergreen. The central area is filled with calcified material, the lighter masses representing the unstained parts, while the black granules indicate the portions of the deposit which were red in the original. x 10.

Fig. 2. — Drawing of a cleared cataractous lens from a young rat, which had been fed with madder during the last six days of life. The granules and masses shown in black were seen as red in the preparation, and represent the vitally-stained portions of the calcareous deposit. x 25.

Fig. 3. — Photograph of a section through a cataractous lens (partner of that shown in Fig. 2) stained with Mallory's connective tissue stain. The lens fibers are represented by the black mass in the center. Around the periphery are seen aggregations of black granules; these are stained red in the original and represent the vitally-stained calcium salts deposited during the period of madder-feeding. x 25.

Fig. 4. — Photograph of anterior aspect of two rings from the trachea of a rat fed daily for five months from birth with madder. Cleared specimen. The reticular structure shown in black is red in the original, and represents the intercellular calcium salts deposited and stained during life. x 35.

Fig. 5. — Drawing from section through a tracheal cartilage similar to that of Figure 4. The dense black areas represent madder-stained calcium salts, and the gray reticular structure is the cartilaginous matrix. x 105.



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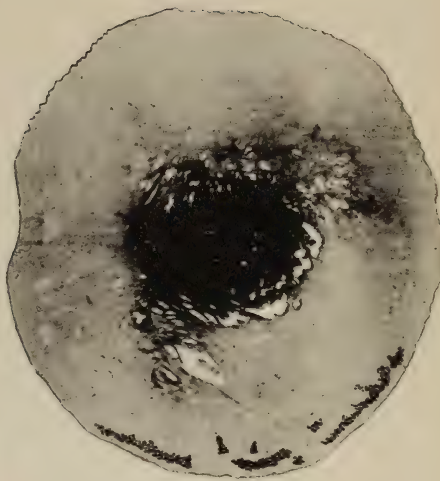

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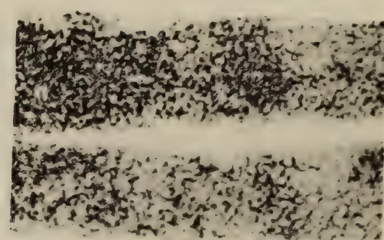

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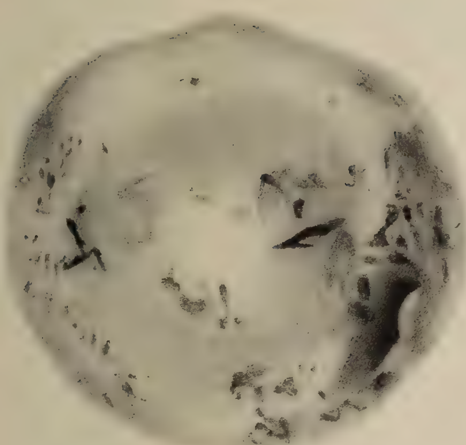

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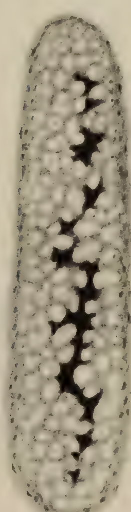

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Macklin. Calcification.

Studies on the Paratyphoid-enteritidis Group.*

IV. The differentiation of the members of the paratyphoid-enteritidis group from B. typhosus with special reference to anaerogenic strains and observations on the fermentative characteristics of the avian types.

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Although the majority of the members of the paratyphoid-enteritidis group are readily differentiated from the typhoid bacillus by their ability to produce gas from glucose, the isolation of an increasing number of strains (Moore,[1] Dorset,[2] Bock,[3] Oette,[4] Wagner,[5] Ohno[6]) which, though failing to produce gas, must be included in this group, makes necessary a basic biological group characteristic that will separate anaerogenic as well as aerogenic types from B. typhosus. Ten Broeck[7] and others have also shown that the ability to produce gas from glucose may vary or even be suppressed. Of particular interest is the anaerogenic B. sanguinarium (Fowl Typhoid, Moore). Smith and Ten Broeck[8] have shown that this organism has a very close cultural and immunological relationship to B. typhosus, although differing in its lack of motility and further separable by agglutinin absorption. They also showed that B. pullorum (Rettger), although it normally produces gas from glucose, is also closely related immunologically to B. typhosus and to B. sanguinarium as well.

In our study of the aerogenic members of the paratyphoid-enteritidis group we found that rhamnose was uniformly and promptly fermented, whereas the typhoid bacillus did not attack this carbohydrate. These observations confirm the results of previous workers, who, as a rule, however, seem to have attributed no special practical importance to these findings. Thus, Uhlenhuth and Hubener[9] in their summary

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give the fermentation of rhamnose as a characteristic of the paratyphoid-enteritidis group, but state that the fermentation (gas production) of glucose alone suffices to separate this group with certainty from B. typhosus. Oette,[4] however, in studying the non-gas-producing "B" type he isolated, showed that it was related culturally to B. paratyphosus and not to B. typhosus, because of its ability to ferment rhamnose. Wagner,[5] in a study of two strains, made the same observation. Both these authors give data indicating that gas production is subject to variation in the human body. We have extended the study of rhamnose fermentation to the available anaerogenic types, especially B. sanguinarium, and to the aerogenic B. pullorum. Other carbohydrates were included, primarily to elicit, if possible, further differences among the three closely related types: B. typhosus, B. sanguinarium, and B. pullorum.

The medium employed was a peptone water containing one per cent of Witte's peptone, .5 per cent of salt, one per cent of the Andrade indicator, and one per cent of the carbohydrate. Unless otherwise stated, acid production alone was observed. The fermentative results are given in Table I.

TABLE I.

	Gas from Glucose.	Acid Production (Number of Strains Studied and Day of Evident Fermentation).								
		Mannite.	Maltose.	Xylose.	Sorbite.	Dulcite.	Arabinose.	Rhamnose.	Inosite.	Adonite.
B. typhosus (37 strains)	—	+	+	29 + 1 + 5 — 9 5 + 8 — 10 2 + 11 — 13	36 + 1 + 2	2 + 5 — 7 6 + 8 — 10 16 + 15 — 21 5 —	34 — 2 † — or +	—	—	—
B. sanguinarium No. 180 . . . / No. 203 . . . / No. 204 . . . / No. 205 . . . / No. 206 . . .	—	+	+	+ 1 — 2	(See notes.) + 9 — 14 | + 9 + 11 | + 14 — 14 | — 31 + 8 | + 14	+ 1 — 2	+	+ 2 — 5	—	—
B. pullorum (13 strains)	+	+	— 14 *	+ 1 — 2	+ 2 — 7	—	+	+	—	—
B. suis (Dorset strain)	—	+	+	+	+	+ or — **	—	+	—	—
B. suis No. 152	—	+	+	+	+ 2	+ or — **	—	+	—	—
B. suis (Ten Broeck strains) . .	—	+	+	+	+	+ or — **	—	+	—	—
" " " " " . .	+	+	+	+	+	+	—	+	—	—
B. paratyphosus No. 93	—	+	+	+	+	+	—	+	—	—
Control Strains. B. paratyphosus "A" . .	+	+	+	—	+	+	+	+	—	—
Control Strains. " " "B" . .	+	+	+	+	+	+	+	+	+	—
Control Strains. B. enteritidis	+	+	+	+	+	+	+	+	—	—

Explanation of Symbols: + = evidence of fermentation within 24 hours. Figure following + is interval in days before positive fermentation was observed. Figure preceding + refers to number of strains. Negative results (—) mean observation for 21 days unless otherwise noted.

Notes: † These two strains (recent isolations) discussed below. * Later observations given in text. ** Irregular, negative, or delayed positive results. Sorbite: duplicate observations with B. sanguinarium.

Source of Cultures: B. sanguinarium No. 180 is Smith and Ten Broeck II. c. We are indebted to Dr. K. F. Meyer for the other four strains. The B. pullorum strains were obtained from Drs. Smith, Rettger, and Meyer. The strains B. suis No. 152 and B. paratyphosus "B" No. 93 are stock strains, the origin of which is not ascertainable. The strains of B. typhosus are, with four exceptions, recently isolated fecal strains.

The time of appearance of acid production is noted, as we found that differences in avidity as well as variability due to the enhancement of low or latent avidity were significant in correlating the relationships of the various groups and of practical importance in their separation. This is best brought out by discussing the more essential carbohydrates employed.

Xylose is fermented by all members of the paratyphoid-enteritidis group with the exception of B. paratyphosus "A." B. typhosus is more closely related to the latter and this correlates with the fluctuating avidity shown by different strains for this carbohydrate. It is suggestive that B. sanguinarium and B. pullorum, closely related to B. typhosus, do not show a maximum avidity.

Dulcite is slowly fermented by the majority of strains of B. typhosus, although it is usually stated that this carbohydrate is not so fermented. Penfold[10] has noted this ability to ferment dulcite slowly, and by cultivation on dulcite media obtained strains which fermented promptly. As has been noted by many observers, there is a gradient avidity for dulcite in the paratyphoid-enteritidis group, B. paratyphosus "A" attacking the carbohydrate slowly, B. paratyphosus "B," and B. enteritidis fermenting more promptly, the latter in general showing the greatest avidity. The intermediate avian strain B. pullorum, and some B. suis strains, break the gradation from B. typhosus to B. enteritidis. B. sanguinarium and B. pullorum differ in relation to this carbohydrate. The anaerogenic B. suis types show a fluctuating avidity for dulcite. It is interesting to note the differences between the two Ten Broeck[7] strains in this respect; apparently the loss of gas production is associated with a partial suppression of the ability to ferment dulcite.

Arabinose was fermented in some tests by two strains of B. typhosus, T. 9 and T. 38. Both show a further peculiarity in that they are unusually sensitive to the action of brilliant green. Further observations have shown that this latent ability to ferment arabinose can be considerably enhanced. The stock culture of T. 9, which gave a positive result in seven days in one test and remained negative for four weeks

in two other tests, was plated and six fishings isolated. One of these fermented in three days, the other five were negative after three weeks. The positive fermentation tube of the original culture was similarly plated, and of six fishings four were positive in twenty-four hours, one in nine days, and the other remained negative after twenty-six days. Evidently growth in the arabinose medium was followed by adaptation, or more probably by selective multiplication of the individuals more capable of fermenting this carbohydrate. All the fractions were typical agglutinatively. The other strain, T. 38, showed a similar adaptation, but the positive results were much slower in developing. Similar results have been noted by Penfold.[11] He found that strains of B. typhosus fermented arabinose slowly and irregularly, but by sub-culture in arabinose media a promptly and regularly fermenting strain could be produced. Some strains of typhoid, therefore, normally possess the ability to ferment arabinose, but their avidity for the sugar is low and appears irregularly. Other strains probably all possess a latent ability which could be developed by appropriate means. From the practical standpoint, however, this does not detract from the differential value of this carbohydrate in separating B. sanguinarium from B. typhosus, as the former ferments promptly and uniformly.

Sorbite is promptly fermented by B. typhosus and by most of the paratyphoid group, which agrees with the findings of other observers. The intermediate avian types show their relationship in the tendency to suppression of this ability. B. pullorum, although fermenting uniformly, does so slowly. B. sanguinarium is not fixed in relation to this fermentation, and the results resemble to some extent those of B. typhosus on arabinose, dulcite, and xylose. Without due regard to the question of low or latent avidity, differential value could be attributed erroneously to such findings. The temporal differences in fermentation between B. typhosus and B. sanguinarium are, however, of differential value.

Maltose fermentation, as noted by Smith and Ten Broeck,[8] differentiates between B. sanguinarium and B.

pullorum. Our results are the same. With prolonged incubation, however, the latter show irregular evidences of fermentation in from sixteen to forty days. As this sugar is notably subject to hydrolysis, the prolonged incubation rather than a latent ability to ferment may explain these findings.

Rhamnose fermentation evidently differentiates B. typhosus from both the aerogenic and anaerogenic paratyphoid types. Rhamnose has been frequently referred to as "isodulcite." As it is a methylated pentose, this term, indicating an alcohol structure, is inappropriate and confusing. B. sanguinarium, however, shows its close relationship to B. typhosus in the lower avidity exhibited for this sugar. The ability to produce gas from rhamnose was also observed with some strains. This was found to be irregular and unreliable, and differences in gas production without significance. Although some observers have induced B. typhosus to ferment rhamnose, from our own observations we feel that for all practical purposes the differentiation given is fundamentally valid. Muller [12] described the formation of fermenting papillæ showing acid change of the indicator on typhoid colonies when grown on rhamnose-agar and considered this a mutation specific for the organism. Penfold [10] observed acid production by typhoid strains when grown in peptone water for seven to twenty-one days, which results are not in accord with our observations.

With the apparently basic characteristic, acid production from rhamnose, we may tentatively define the limits of the paratyphoid-enteritidis group. To be included, an organism must produce acid from rhamnose, but not from salicin, saccharose or lactose, the ability to produce gas from glucose being a secondary characteristic.

In regard to B. pullorum and B. sanguinarium, which are of interest because of their close antigenic relationship to B. typhosus, the differences can be summarized as follows (Table II.). The time limits for differentiation are noted where these differences are temporal only, and the rapidity

with which the difference is elicited, where the difference is qualitative:

TABLE II.

Type.	Gas from Glucose.	Acid Production from				
		Maltose (prompt).	Dulcite (48 hrs.).	Arabinose (prompt).	Rhamnose (slow).	Sorbite (48 hrs.).
B. typhosus.....	—	+	—	—	—	+
B. sanguinarium.	—	+	+	+	+	—
B. pullorum	+	—	—	+	+	+ or —

Gas production by B. pullorum may be suppressed, as shown by Smith and Ten Broeck.[8] We are indebted to them for the strain which they found did not produce gas from glucose. In our hands this strain has resumed its ability to produce gas, showing the tendency to variation in this characteristic. The differences in maltose fermentation given in the table, as has been stated, were observed by them. Since the completion of our work Rettger and Koser[13] have reported a comparative study of B. sanguinarium and B. pullorum and found that dulcite, and also dextrine, separates these two types. B. typhosus was not included in their study.

We were led to repeat the agglutinative observations of Smith and Ten Broeck, as the agglutinative similarity of B. typhosus, B. sanguinarium, and B. pullorum, associated with marked cultural differences, parallels our findings[14] with other members of the paratyphoid group, some of which showed equally marked cultural differences with apparent agglutinative identity. The results are summarized (Table III.). Only one dilution is recorded, the highest in which equally complete reactions were found for the three types.

TABLE III.

Type.	Typhoid Serum (Horse).	Fowl Typhoid Serum (Rabbit).	B. Pullorum Serum (Rabbit).
B. typhosus	6,000	1,250	1,000
B. sanguinarium	800	1,500	1,000
B. pullorum	800	1,500	1,250

In general, the results are similar to those of Smith and Ten Broeck as well as to those of Rettger and Koser. Our results with typhoid serum, however, differ from those of the former observers in the very distinctly lower cross-agglutination of B. sanguinarium and B. pullorum. Because of this difference two other horse sera were tried with the same results. The differences noted, however, with our sera were paralleled by the absorption results of Smith and Ten Broeck, who found that the avian strains would not remove the specific agglutinins for B. typhosus from typhoid sera. The high degree of cross-agglutination recorded by these workers is not, therefore, constant with all sera, but if present, absorption methods will still differentiate.

The other anaerogenic strains also showed no agglutinative differences, although as is evident in Table I., they differed somewhat among themselves in their fermentative reactions and sharply from the type strain of B. paratyphosus "B" included as a control. The agglutinative results (macroscopic method) are as follows:

Strains.	Sera and Dilutions.						
	B. paratyphosus A.		B. paratyphosus B.			B. enteritidis.	
	1-1,000	1-5,000	1-2,000	1-4,000	1-7,000	1-1,000	1-5,000
B. paratyphosus B. No. 93,	−	−	+++	++	++	−	−
B. suis (Dorset)	−	−	+++	++	++	−	−
B. suis No. 152	−	−	+++	++	++	−	−
Ten Broeck:							
Gas −	±	−	++++	+++	++	−	−
" + ...	±	−	+++	+++	++	−	−
Controls:							
A type	++++	++	−	−	−	−	−
B type	−	−	++++	+++	++	−	−
B. enteritidis	−	−	−	−	−	++++	+++

++++ Complete agglutination.

SUMMARY. — The ability to produce acid from rhamnose is the essential characteristic of the paratyphoid-enteritidis group, differentiating both the aerogenic and anaerogenic members from B. typhosus. Additional cultural differences between B. typhosus, B. sanguinarium, and B. pullorum are presented. The agglutinative relationship of the strains studied is recorded. Observations are added on the low or latent avidity for carbohydrates in relation to variability and practical differentiation. Without due regard to these factors, erroneous differential significance might easily be given to variation even among members of the fixed groups.

REFERENCES.

NOTE. — An acknowledgment is due Dr. W. W. Ford, who called our attention to his paper, "The Carbohydrate Reactions of the Paratyphoid or Paracolon Group. (Preliminary Communication)," Medical News, June 17, 1905. He determined gas production from a series of carbohydrates by members of this group. Although some of his conclusions as to inability to ferment, being based on gas production only, are not valid, two striking results were obtained. He noted that his "A" and "B" strains differed on xylose. He, therefore, was the first to note this difference. Unaware of his findings, our results with a large series of cultures constitute an independent confirmation. He also found that his "hog-cholera" strains failed to produce gas from arabinose and suggested that

this was of differential value in separating this group. Various workers have employed this carbohydrate and although differences have been noted no similar significance has been attributed by them to these findings. It is of interest to note that the differential value suggested by Ford has been found to hold for many, but not all of the "suis" strains we have studied, employing acid production, however, as indication of fermentation. His results with xylose have been overlooked, not only by us, but by all who have worked on this group of bacteria during the last ten years. Evidently, had it been noted and repeated, the significance of differences in xylose fermentation would soon have been generally known.

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A SELECTIVE MEDIUM FOR THE ISOLATION OF B. PESTIS FROM CONTAMINATED PLAGUE LESIONS AND OBSERVATIONS ON THE GROWTH OF B. PESTIS ON AUTOCLAVED NUTRIENT AGAR.*

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In the routine examination of dead rats for plague, specimens are often received that are too badly contaminated to permit of the isolation of plague bacilli by plating the spleen or bubo upon ordinary nutrient agar, and yet the inoculation of this material into guinea-pigs produces typical plague lesions from which plague colonies in almost pure culture are obtained on nutrient agar plates. If a selective medium for plague bacilli could be devised that would inhibit the growth of the contaminating bacteria to such an extent that the plague bacilli would not be overgrown by these organisms, a final diagnosis of plague could be rendered several days sooner; for, in the instance cited above, the plague bacilli would have to be subjected to cultural and agglutination tests after the death of the inoculated guinea-pig before a positive diagnosis of plague would be justified, whereas, if the plague bacilli had been isolated by direct plating of the contaminated material from the rat upon a selective medium, these tests might have been performed before the death of the guinea-pig. A saving of two or three days in the time required for a definite diagnosis is obviously of great importance in the practical application of preventive measures.

Furthermore, it seemed probable that a selective medium might occasionally lead to positive results when the rat material yielded negative results upon inoculation into guinea-pigs, owing to contamination with bacteria pathogenic for these animals.

The recent studies of the action of various aniline dyes upon

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bacteria other than B. pestis by Churchman[1] and other workers gave us sufficient encouragement to suppose that B. pestis, being a Gram-negative organism, would probably in regard to the effect of these dyes upon its growth belong to the same class as B. prodigiosus and others, the growth of which is not inhibited by the basic dyes, while that of the organisms of the Gram-positive group is. Just here a cursory review of Churchman's work may not be out of place. In 1912 he discovered that Gentian violet has an inhibitory action on the growth of B. subtilis, while not affecting that of B. prodigiosus, and he came to the conclusion that bacteria may, on this account, be divided into two classes, viz., those violet-positive and those violet-negative, the former corresponding to the Gram-positive organisms and the latter to the Gram-negative. This action was the same, whether the dye was used for vital staining of the bacteria or was added to the media upon which they were grown, except that he found that the effect of the dye is not quite so constant when it is applied to the bacteria themselves, as when it is incorporated with the media. To prove that it is the Gram-positive organisms that are inhibited and the Gram-negative that are not, he used cultures of S. p. aureus, S. p. albus, B. diphtheriæ or B. anthracis, Sar. rosea, Streptothrix actinomyces, blastomycetes, and certain yeasts, and he found that the growth of these is inhibited by Gentian violet, thus placing them in the same class as B. subtilis, while the Gram-negative organisms, such as B. pyocyaneus, B. typhosus, B. paratyphosus, B. coli, etc., are all Gentian violet-negative, and thus belong to the same class as B. prodigiosus. He used the Gentian violet in a dilution of 1/1,000, and in some experiments as strong as 1/700, and designated organisms as violet-positive or violet-negative according to whether they were or were not inhibited in their growth by the stain. Simon[2] in a later article in 1914 criticises this division, as he considers that the violet color has really nothing to do with the inhibitory character of the stain; but he admits that certain organisms are inhibited by certain dyes, while others are not. On the other hand, Churchman[1] considers this division into

violet-positive and violet-negative organisms as more certain than that of Gram-positive and Gram-negative. There are according to the latter author a few Gram-positive organisms, such as B. Welchii, whose growth is hindered, but not completely inhibited, save in extremely strong dilutions. These are B. sporogenes, an occasional strain of Streptococcus and of Pneumococcus, and most of the acid-fast group. There are also a few of the Gram-negative bacteria, which do not follow the general rule. However, we find at times that organisms which are usually Gram-positive have become Gram-negative. This fact has been attributed to the age of the strain, an old culture becoming Gram-negative. Churchman[1] found that occasional strains varied in this respect with regard to the inhibitory action of dyes also; but for the vast majority of bacterial species the rule holds good, and he contends that the age of the organism does not affect the action of the dye.

Neither Churchman,[1] nor Krumwiede and Pratt,[3] nor Simon[2] included the plague bacillus in the list of bacteria upon which they studied the action of various stains incorporated in nutrient agar. It seemed therefore of interest to carry out such experiments with B. pestis; especially so since these experiments would indicate which stains should be used in the attempts to devise a selective medium for this organism.

The nutrient agar employed in this laboratory is prepared as follows: One pound of beef-heart is passed through a meat-chopping machine and soaked in one liter of distilled water over night in the ice-box. The fluid is squeezed through cheesecloth, heated to boiling, and filtered through filter paper. One per cent peptone, one-quarter per cent of sodium chloride, and one and one-half per cent of agar-agar are added; four cubic centimeters of a normal NaOH solution are added to prevent a too high degree of acidity, and the mixture is heated for thirty minutes in the autoclave at fifteen pounds pressure. The medium is titrated to +1 (hot titration), cleared with egg-white, filtered through cotton and stored in flasks in two hundred-cubic-centimeter

amounts. These are sterilized by being heated in the autoclave for twenty minutes at fifteen pounds pressure.

All our plague cultures yielded an abundant growth on slants prepared from this medium when transplants were made by spreading one loopful of the growth over the surface of the slant.

In working with nutrient agar containing stains the dosage of the bacteria inoculated constitutes an important factor in determining the limits of inhibition and, in order to obtain accurate and constant results, it has been the custom in this laboratory to so gauge the bacterial suspension used that one loopful yields between thirty and one hundred colonies. In the experiments with B. pestis and Staphylococcus described below larger doses were employed. Slight inhibition is then usually indicated by a reduction in the number of colonies and in the size of the colonies that do develop — complete inhibition by the absence of growth. When experiments of this nature were undertaken with our plague cultures we were surprised to find that no colonies developed on any of the plates, not even on the controls. In other words, if a mass of plague bacilli are inoculated upon our nutrient agar an abundant growth develops, but if isolated plague bacilli are scattered over the surface of the agar they do not grow into colonies.

Jacobson [4] describes a bacillus (Bacterium typhi mutabile) belonging to the typhoid-colon group which showed the peculiarity of not growing upon Conradi-Drigalski agar, by which method he attempted to isolate it from patients suffering from typhoid fever during an epidemic in Denmark, from November, 1908, to 1909. He ruled out the crystal violet of this medium as being the inhibiting agent and was forced to come to the conclusion that the inhibition was due to some substance produced in the medium by the process of sterilization in the autoclave. This variety grew as well on Endo and Padlewski media as the usual strains of B. typhosus. He tried many different forms of agar, thinking to eliminate the cause of the inhibition, but finally decided that the addition of sodium sulphite was all that was

necessary. The oftener that the agar was sterilized the greater was the inhibition for this organism.

Fromme[5] also encountered a strain of B. typhosus isolated at autopsy that gave very small colonies on Conradi-Drigalski agar, but colonies of the usual size on Endo agar. He, too, thought that the toxic substances in the agar were produced during sterilization by heat. Jacobson[4] found that heating in the autoclave for more than fifteen minutes was necessary to call forth the inhibition of his B. typhi mutabile, but heating at 100° C. sufficed for the inhibition of Fromme's strain. Fromme[5] observed that the addition of .25 per cent sodium sulphite to the agar yielded the best growth, but good-sized colonies appeared with one per cent to .1 per cent of sodium sulphite.

It seemed probable that the same substances in the autoclaved agar that were responsible for the inhibition of the growth of Jacobson's strain of B. typhosus were also concerned in the inhibition of the plague bacilli in our experiments. If this were true, then the addition of sodium sulphite should render the autoclaved nutrient agar favorable for the development of colonies of plague bacilli. We have repeated this experiment many times, using different lots of agar, with uniform results. The plague bacilli develop into colonies regularly on the nutrient agar plus sodium sulphite, but do not develop on the same autoclaved agar without the sodium sulphite. Jacobson[4] used .025 per cent sodium sulphite; we found that much smaller amounts were equally effective. As a routine procedure, for example, in the experiments with stains described below we have employed .025 per cent, but we have shown that .0125 per cent is also effective and in one experiment there was only slight inhibition after the addition of .00625 per cent of sodium sulphite. We found also that .05 per cent sodium sulphite is effective if added to the melted agar, cooled to 45° C. immediately before the plates are poured, or if heated fifteen minutes with the agar at 100°, or finally if heated with the agar in the autoclave at fifteen pounds pressure for thirty minutes.

TABLE I.

	Meat Infusion Agar + 1/20% Na_2SO_3.			Same without Na_2SO_3.
	Heated ½ hour at 120° C.	Heated ¼ hour at 100° C.	Not Heated.	
B. pestis No. 3202 (ground squirrel)	+	+	+	—

The fact that sodium sulphite converts the agar into a better culture medium for plague bacilli, even if the agar is autoclaved after the addition of the sulphite, suggests that it might be well to add this salt as a routine procedure to all nutrient agar immediately before sterilization in the autoclave; for, if by this procedure the agar is rendered so much more favorable for the plague bacilli, it is probably made somewhat more favorable for the growth of most other bacteria. Furthermore, it is likely that heating in the Arnold sterilizer on three successive days produces the same toxic substances as autoclaving, though perhaps in smaller amounts, and it would therefore seem advisable to add .025 per cent sodium sulphite in this instance, also immediately before beginning the sterilization.

Even after the addition of sodium sulphite the plague colonies did not grow to a fair size until after forty-eight hours' incubation at 30° C.

TABLE II.

B. Pestis.	Meat Infusion Agar.					
	Agar + 1.	Agar + .5.	Agar ± 0.	Agar + 1 + Na_2SO_3.	Agar + .5 + Na_2SO_3.	Agar ± 0 + Na_2SO_3
Squirrel No. 3202,	—	—	—	+	+	+
" No. 3199,	—	—	—	+	+	+
Rat No. A	—	—	—	+	+	+
" No. B	—	—	—	+	+	+
" No. C	—	—	—	+	+	+

This table has been introduced here to show that with five different strains of B. pestis and with three different reactions of the meat-infusion agar there is no growth whatever of the plague bacilli except where sodium sulphite has been added.

The above preliminary experiments, showing the interesting fact that our nutrient agar is rendered much more favorable for the development of plague bacilli by the addition of sodium sulphite, indicated clearly that this salt should be added to the agar in studying the influence of various dyes upon the growth of plague bacilli. We selected .025 per cent as an appropriate strength of the salt. Since sodium sulphite decolorizes and renders inert certain of the basic stains, we decided to prepare parallel plates of each stain in the same agar with and without sodium sulphite and to inoculate them with the S. p. aureus in order to determine the influence of the small amount of the sodium sulphite present upon each of the stains. The S. p. aureus apparently grows as well upon the plain agar without the sodium sulphite as with it; hence, if there is greater inhibition with a given amount of a stain in the plain agar than in the sodium sulphite agar, some of the stain in the latter medium must have been rendered inert by the sodium sulphite. Furthermore, since the staphylococcus is a representative of those Gram-positive organisms that are readily inhibited in their growth by most of the basic stains, Table III. shows in a general way the relative toxicity of each stain for plague and for those organisms.

TABLE III.

Stain.	B. Pestis.	Staphylococcus.	
	Meat-Infusion Agar.	Meat-Infusion Agar.	
	Plus Na_2SO_3 1/40%.	Plus Na_2SO_3 1/40%.	Without Na_2SO_3.
Crystal violet	1/400 — 1/600 ± 1/700 +	1/20,000 — 1/50,000 ± 1/100,000 +	1/20,000 — 1/50,000 ± 1/100,000 +
Methyl violet B. . .	1/20 ± 1/40 ± 1/80 +	1/8,000 — 1/10,000 +	1/12,000 — 1/20,000 +
Methyl violet 6 B. .	1/200 — 1/400 +	1/10,000 — 1/25,000 ± 1/50,000 +	1/25,000 — 1/50,000 ± 1/100,000 +
Hoffman violet . . .	1/20 — 1/50 +	1/20,000 — 1/40,000 +	1/20,000 — 1/40,000 ± 1/80,000 +
Gentian violet . . .	1/20 — 1/40 ± 1/80 +	1/5,000 — 1/8,000 ± 1/10,000 +	1/8,000 — 1/16,000 ± 1/20,000 +
Ethyl violet	1/1,000 — 1/2,000 ± 1/4,000 +	1/10,000 — 1/20,000 ± 1/40,000 +	1/10,000 — 1/20,000 ± 1/40,000 +
Brilliant green . . .	1/2,000 — 1/4,000 ± 1/8,000 +	1/100,000 — 1,500,000 ±	1/100,000 — 1/500,000 ±
Malachite green . .	1/20 ± 1/40 +	1/2,000 — 1/5,000 +	1/200,000 — 1,400,000 ±
Iodine green	1/20 ± 1/50 +	1/400 — 1/800 ± 1/1,000 +	1/400 — 1/800 ± 1/1,000 +
Eosin (yellowish) .	1/200 — 1/400 ± 1/1,000 +	1/40 ± 1/80 +	1/20 ± 1/80 +
Basic fuchsin	1/20 +	1/200 — 1/400 +	1/2,000 — 1/4,000 +
Congo red	1/20 +	1/20 +	1/20 +
Saffranine	1/20 +	1/400 — 1/800 +	1/400 — 1/800 ± 1/1,600 +

TABLE III. — *Continued.*

Stain.	B. Pestis.	Staphylococcus.	
	Meat-Infusion Agar.	Meat-Infusion Agar.	
	Plus Na_2SO_3 1/40%.	Plus Na_2SO_3 1/40%.	Without Na_2SO_3.
Bismarck brown	1/20 ± 1/40 +	1/20 +	1/20 +
Night blue	1/20 +	1/2,000 — 1/4,000 ± 1/5,000 +	1/4,000 — 1/8,000 +
Victoria blue	1/1,000 — 1/2,000 +	1/2,000 — 1/4,000 ± 1/8,000 +	1/4,000 — 1/8,000 ± 1/16,000 +
Methylene blue	1/20 — 1/40 ± 1/80 +	1/40 — 1/100 ± 1/200 +	1/40 — 1/100 +
Nile blue	1/20 +	1/4,000 — 1/8,000 ±	1/4,000 ± 1/8,000 +
Toluidene blue	1/20 — 1/40 ± 1/100 +	1/100 — 1/200 +	1/100 ± 1/200 +

— = no growth.
± = more or less inhibition of growth.
+ = no inhibition of growth.
The figures indicate fractions of 1% of the stain in the agar.

In performing the experiments, the results of which are recorded in Table III., the sodium sulphite was first added to the agar in bulk and then varying amounts of the stains to be tested on that day were added to portions of the agar and plates were poured. It is seen from Table III. that the different stains are influenced with regard to toxicity for staphylococci quite differently by the addition of the sodium sulphite to the agar. Crystal violet, ethyl violet, brilliant green, iodine green, and Bismarck brown are unaffected by the sodium sulphite; staphylococci are inhibited by the same amount of the respective stains in the plain agar as in the agar plus sodium sulphite. About twice as much of the second group of stains is required for inhibition in the sodium sulphite agar as in the plain agar; this group

comprises methyl violet B, methyl 6 B, Hoffman violet, Gentian violet, saffranine, night blue, and Victoria blue. The toxicity of basic fuchsin is reduced ten times, and that of malachite green 1A eighty times by the addition of sodium sulphite. Finally, in the case of methylene blue, nile blue, and toluidene blue the toxicity appears to be slightly increased by the addition of the sodium sulphite. We must, therefore, assume that only a portion of the .025 per cent sodium sulphite added is utilized in destroying the toxic substances of the nutrient agar, thus leaving a free remnant that affects the toxicity of the different stains in markedly different degrees.

It is also seen from Table III. that the different stains in sodium sulphite agar are much more toxic for the staphylococcus than for the plague bacillus. For the sake of clearness the multiple indicating roughly this relative toxicity for each of the stains is given in Table IV.

Table IV.

1.	Hoffman violet	800	9.	Brilliant green	100
2.	Nile blue	500	10.	Saffranine	40
3.	Night blue	250	11.	Basic fuchsin	20
4.	Crystal violet	140	12.	Iodine green	20
5.	Methyl violet B	125	13.	Ethyl violet	10
6.	Methyl violet 6 B	125	14.	Victoria blue 4 R	4
7.	Gentian violet	125	15.	Methylene blue	3
8.	Malachite green	125	16.	Toluidene blue	2

Thus Hoffman violet is eight hundred times more toxic for the S. p. aureus than for B. pestis, nile blue is five hundred times more toxic, and so on. Theoretically, the first four stains in the list would be more promising for the preparation of a selective medium for plague bacilli than the other dyes.

Since no naturally-infected plague rats were available in

the vicinity of New York city, we inoculated guinea-pigs cutaneously with plague bacilli, allowed them to die, and kept them in a closed glass jar at room temperature for seven days after death. Bits of the spleen and the bubo were crushed in saline solution and furnished contaminated material for testing the comparative value of the media containing the stains used in the preceding experiments. In a number of different tests plague colonies were readily fished from several of the plates containing agar with stains, when the control plain agar plates showed a spreading growth over the entire surface, rendering it impossible to isolate plague bacilli. Such tests indicated that nutrient agar containing 1/700 per cent of crystal violet is probably the most effective medium of those tried.

We wish to recommend tentatively this medium for the isolation of plague bacilli from the bodies of animals dead for some days of plague, where the contaminating organisms are present in great numbers, realizing, however, that we have had no opportunity to determine the value of the method in an actual epizoötic of plague.

A study of Table III. reveals the fact that Victoria blue and ethyl violet are much more toxic for plague bacilli than the other violet stains. This seemed all the more surprising because these two stains are not particularly toxic for most Gram-negative bacilli. We have determined for six of the stains the maximum amount that permits growth of typhoid bacilli — using the same lot of agar containing .025 per cent of sodium sulphite that was employed in the above experiments with plague bacilli. Table V. shows the maximum percentages of these stains permitting growth of plague bacilli and typhoid bacilli respectively.

TABLE V.

Stain.	B. Pestis.	B. Typhosus.
Methyl violet B	1/80	1/40
Methyl violet 6 B	1/400	1/80
Crystal violet	1/700	1/400
Gentian violet	1/80	1/40
Ethyl violet	1/4,000	1/20
Victoria blue	1/2,000	1/40

It seems probable that this marked toxicity of ethyl violet or Victoria blue for B. pestis might be of practical value in the identification of this organism, but freshly isolated cultures would have to be first tested in this regard and such cultures are not available here.

While we were engaged in the preliminary study of the influence of the addition of sodium sulphite to autoclaved agar an interesting factor accidentally introduced itself. One day it was observed that on a control plate of plain meat-infusion agar of reaction + 1 there was a number of contaminating colonies, and that in the vicinity of these there were a few colonies of B. pestis, while on the non-contaminated portion of the plate there was no growth of B. pestis, as was always the case on the plain agar without the sodium sulphite. The contaminating organism was obtained in pure culture and proved to be a large spore-forming bacillus, Gram-positive, non-motile, non-liquefying, producing acid, but not gas with lactose, dextrose, and saccharose, a strict aerobe producing a wrinkled pellicle in broth with no turbidity of the medium.

Experiments were then carried out as follows: An appropriate amount of an agar slant culture of the organism was suspended in melted nutrient agar cooled to 45° C. and a large loopful of the inoculated agar was streaked across the surface of a sterile Petri dish. When the agar had solidified,

autoclaved nutrient agar melted and cooled to 45° C. was poured into the plate, completely covering the inoculated agar. After the surface of the agar had been dried in the incubator, a loopful of a suspension of plague bacilli in saline solution was spread uniformly over it; after twenty-four hours' incubation there were deep colonies in the streak and no surface colonies; after a further incubation of twenty-four to forty-eight hours plague colonies appeared on the surface of the agar immediately above the growth of the other organism, but not on other portions of the plate. This experiment was repeated by us many times with the same result.

Similar experiments were performed, substituting for the large bacillus in the depth of the agar the following organisms: B. typhosus, B. paratyphosus A, B. paratyphosus B, and S. p. aureus respectively. Plague colonies appeared above the deep growth of each of these cultures except B. paratyphosus B.

Two possible explanations of the phenomenon may be suggested: (1) The bacilli growing in the depth of the agar may produce some substance or substances which diffuse into the neighboring agar and render inert the substances contained in it that inhibit the growth of plague bacilli. The substances produced by the deep growth would thus act similarly to the sodium sulphite. (2) The deep growth may produce certain substances that so favor the growth of plague bacilli that the latter multiply and produce colonies in spite of the presence of inhibiting substances in the agar. The recent work of Lloyd[6] is particularly interesting in this regard. She showed that freshly isolated cultures of the meningococcus require for their growth the presence in the medium of vitamins, or substances the lack of which in the diet call forth beri-beri in human beings and polyneuritis in fowls. It seems possible that the bacilli forming the deep colonies may produce vitamins which diffuse into the adjacent agar and thus render it a much more favorable medium for plague bacilli. That acids or alkalis produced by the

deep growth are not responsible for the development of the plague bacilli is shown by the fact that the latter do not grow on the autoclaved agar when the reaction is rendered more acid or more alkaline (see Table II). Eddy[7] has recently found that pancreatic vitamin acts favorably when fed to infants suffering from malnutrition. Through the courtesy of Dr. A. F. Coca we obtained a sample of Mr. Eddy's pancreatic extract and found that when added to our autoclaved nutrient agar in the amount of ten or twenty per cent the latter allowed plague bacilli to develop into colonies. Whether the pancreatic extract acted through the vitamins or through some other substances contained in it, we cannot say.

Simon[8] observed that certain acid dyes seemed to exert a favoring influence upon the growth of certain bacteria. He was able to offer no explanation of this phenomenon. In view of the favorable action exerted by sodium sulphite upon autoclaved nutrient agar with regard to the development of plague bacilli, we would suggest that possibly the acid stains exert their favoring influence by virtue of the SO_3Na groups which they contain.

Grübler's stains were used exclusively in the above experiments. All cultures of B. pestis were incubated at 30° C., the optimum temperature for this organism.

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